



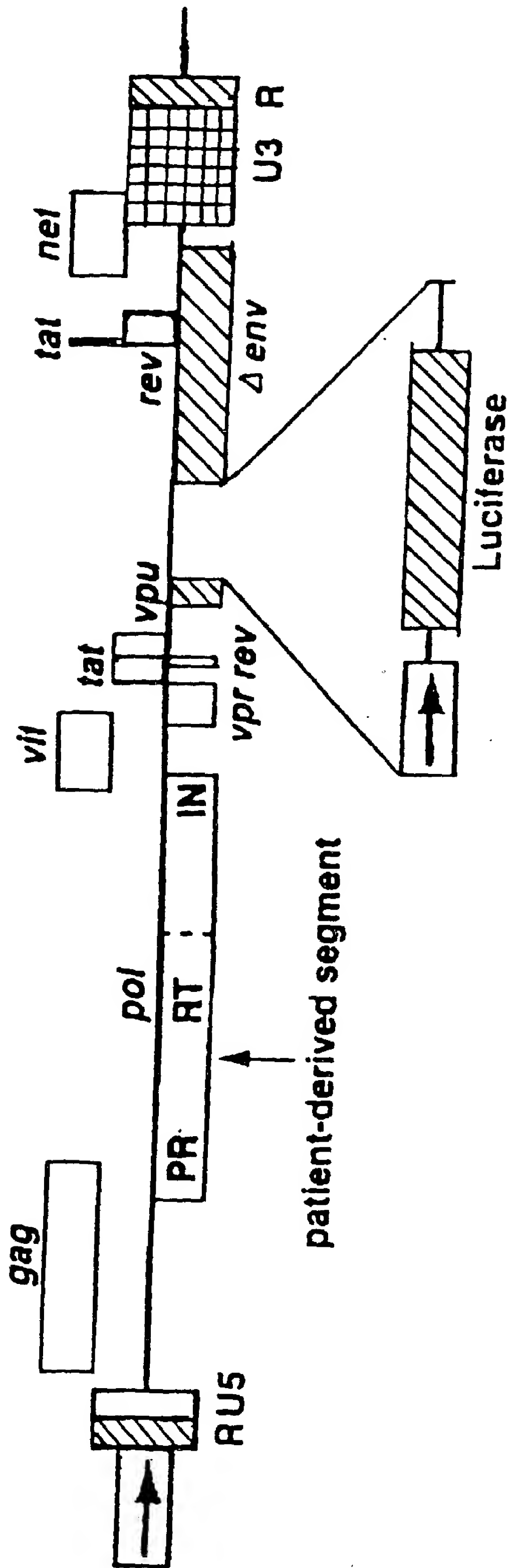
Applicants : Neil T. Parkin and Rainer Ziermann
U. S. Serial No. 09/874,472
Filing Date: June 4, 2001
Title of the Invention: MEANS AND METHODS FOR
MONITORING PROTEASE INHIBITOR
ANTIRETROVIRAL THERAPY AND GUIDING
THERAPEUTIC DECISIONS IN THE TREATMENT
OF HIV/AIDS

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FIGURE 1

PhenoSense™ HIV Resistance Test Vector.





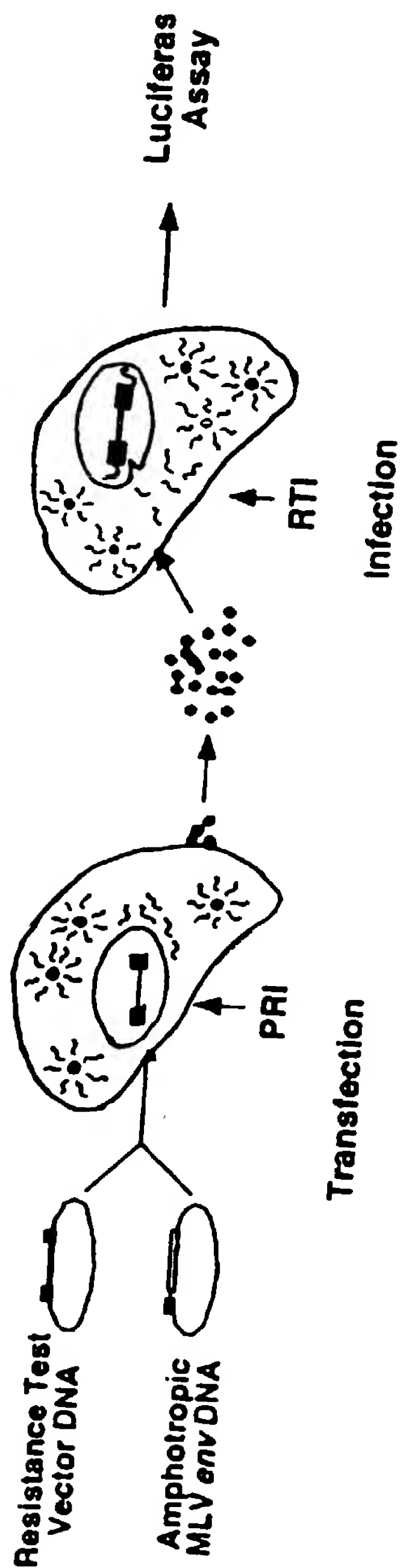
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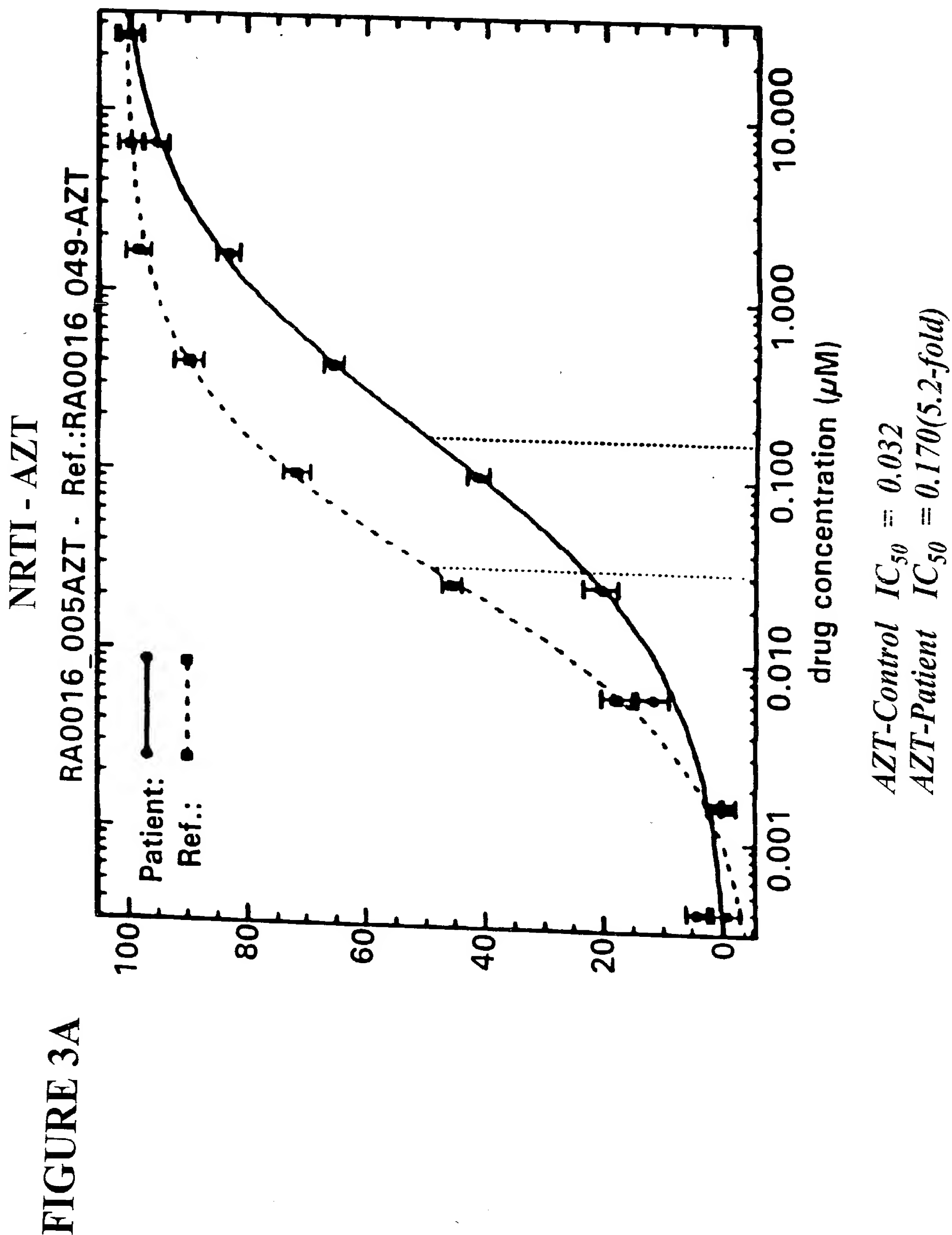
FIGURE 2

PhenoSense™ HIV Schematic Diagram.



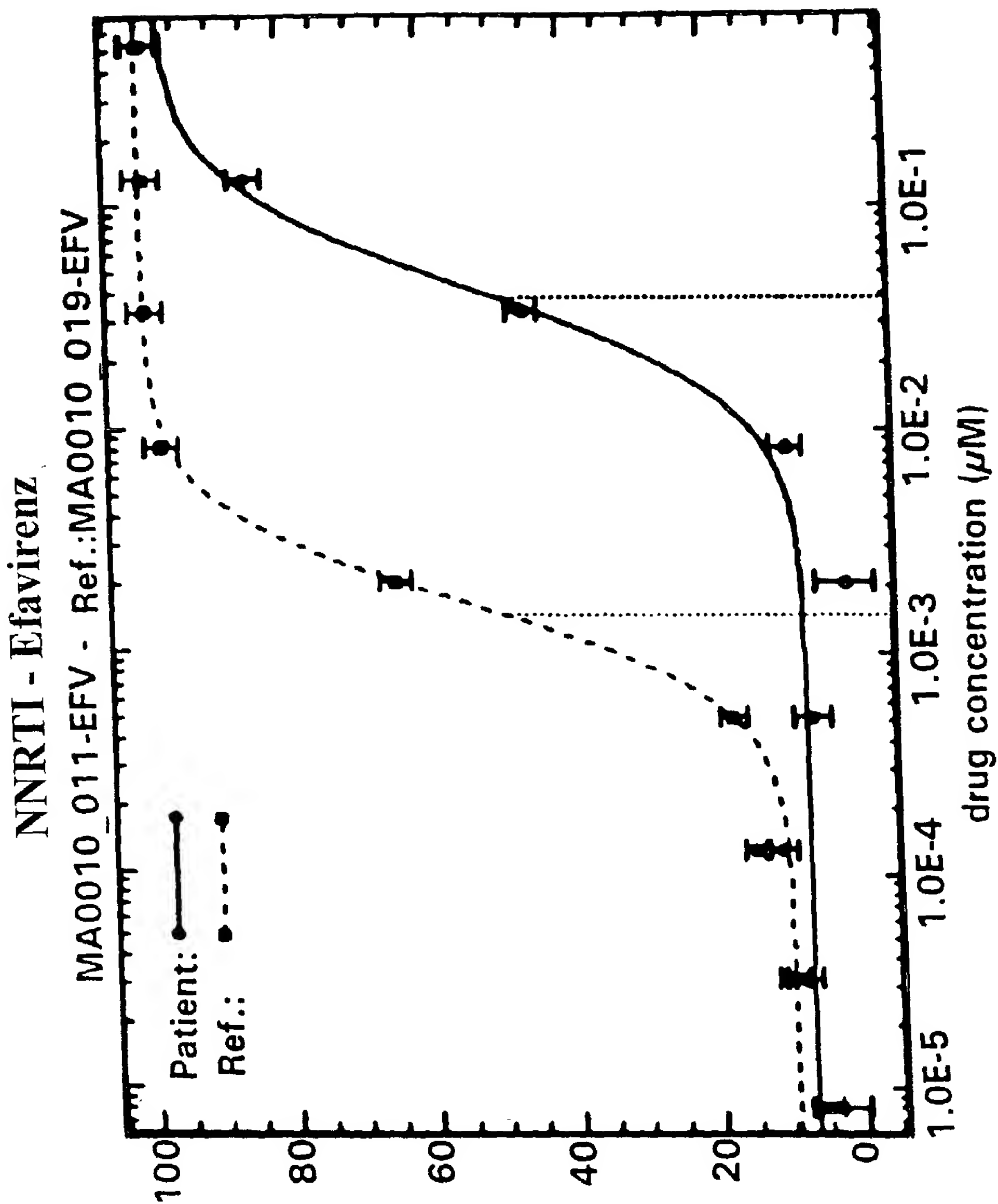


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EFV-Control $IC_{50} = 0.0015$
EFV-Patient $IC_{50} = 0.0380$ (25.6-fold)

FIGURE 3B



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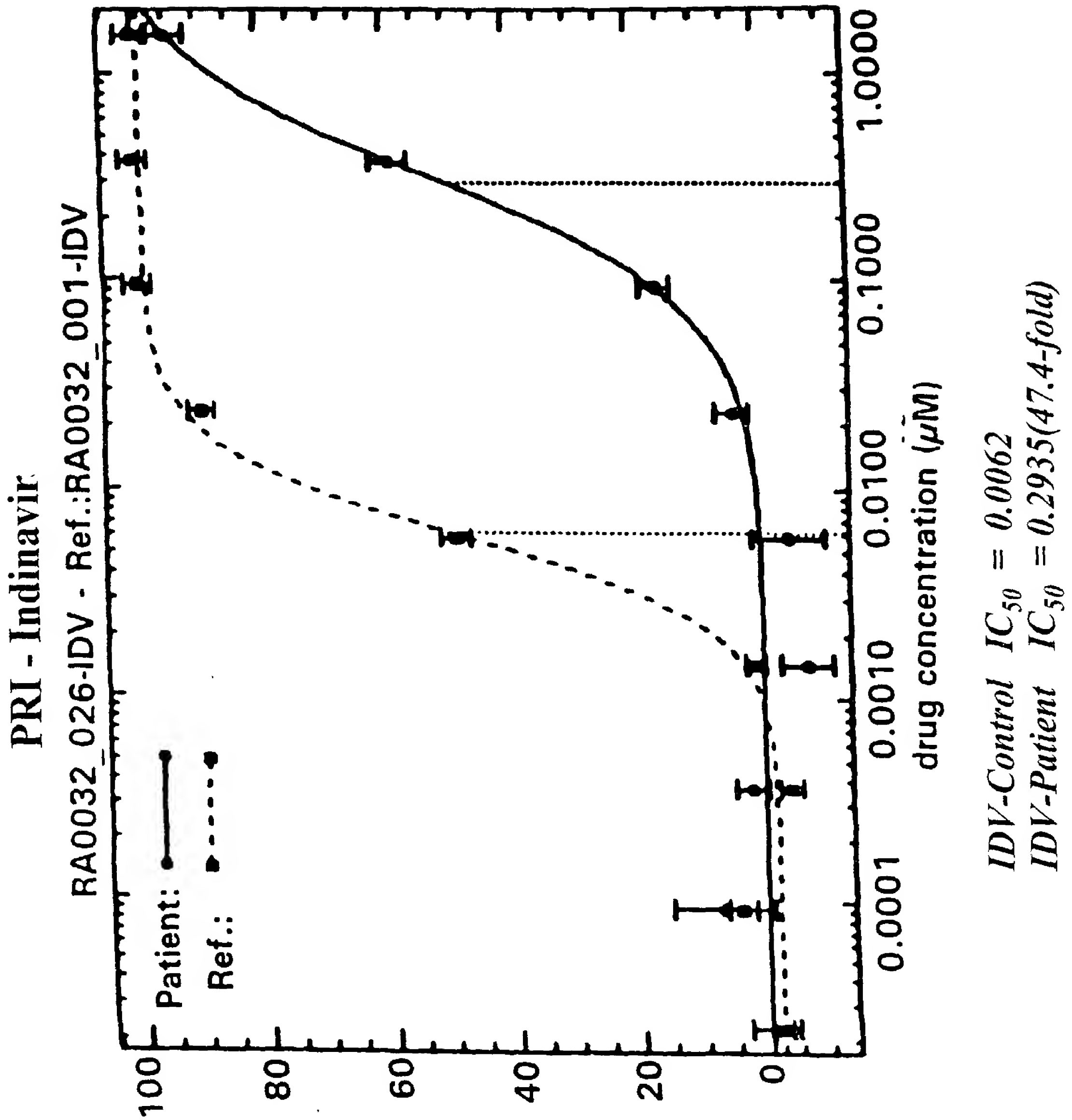


FIGURE 3C



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FIGURE 4A

SQV

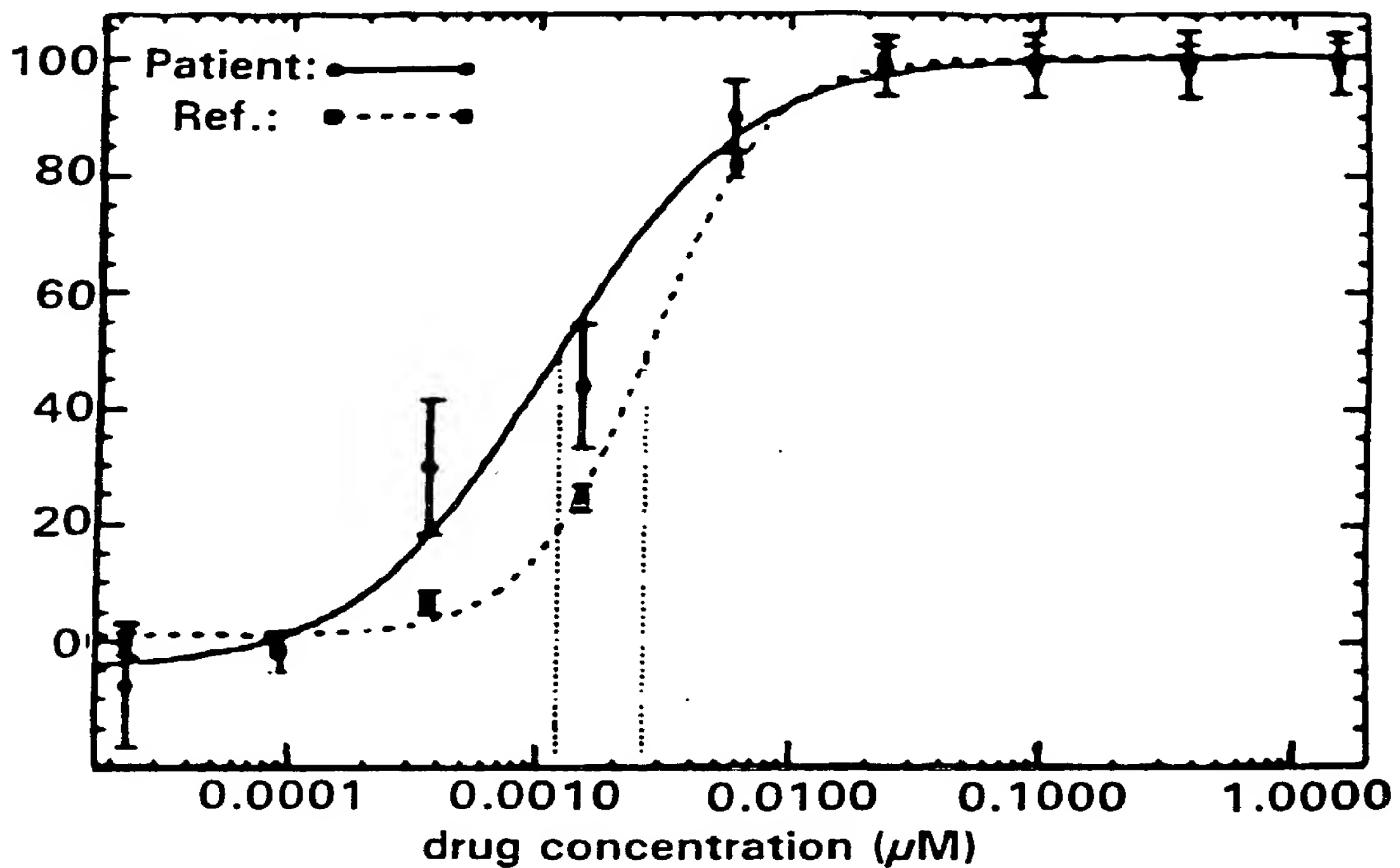
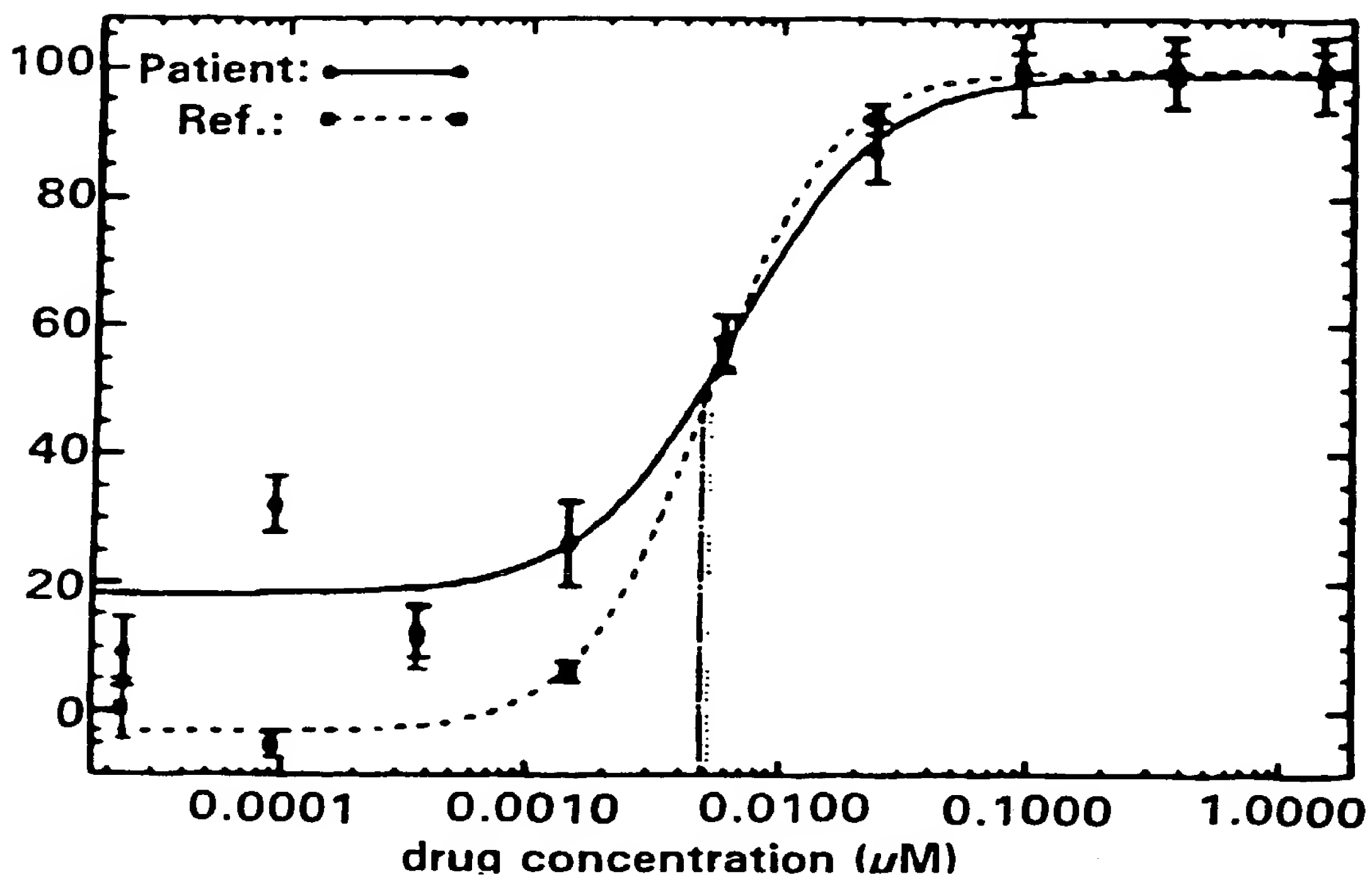


FIGURE 4B

IDV





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FIGURE 4C

RTV

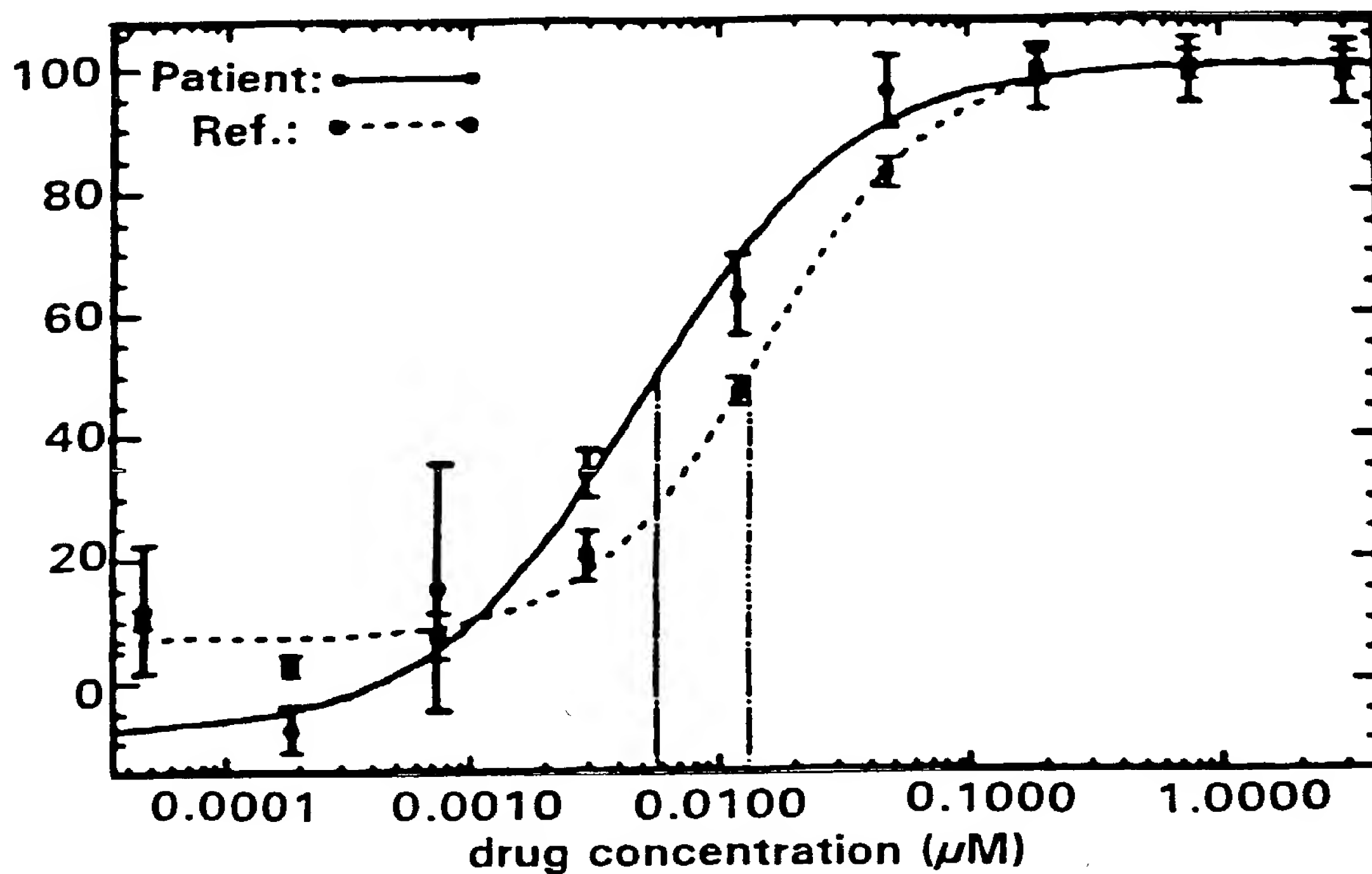
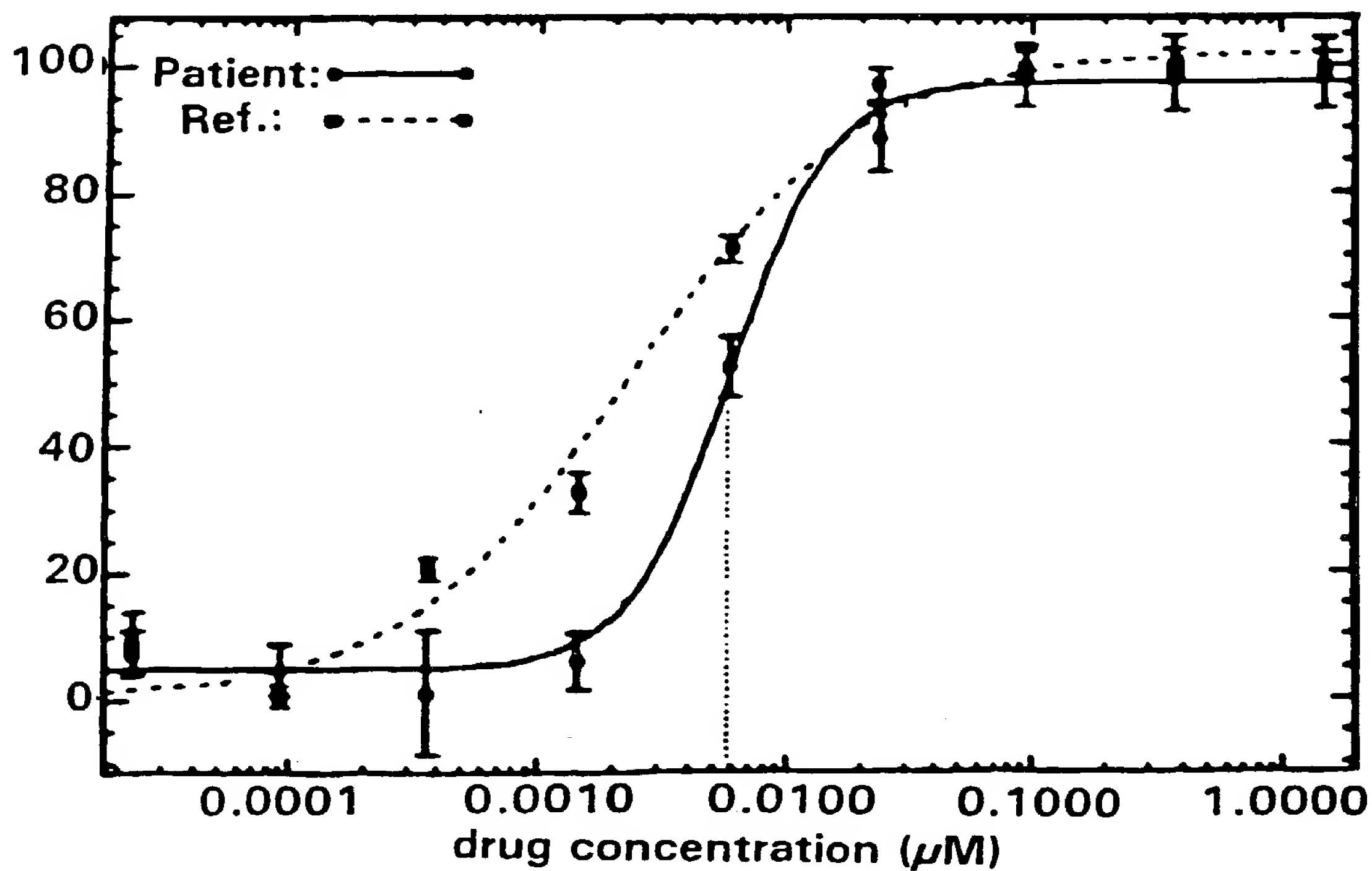


FIGURE 4D

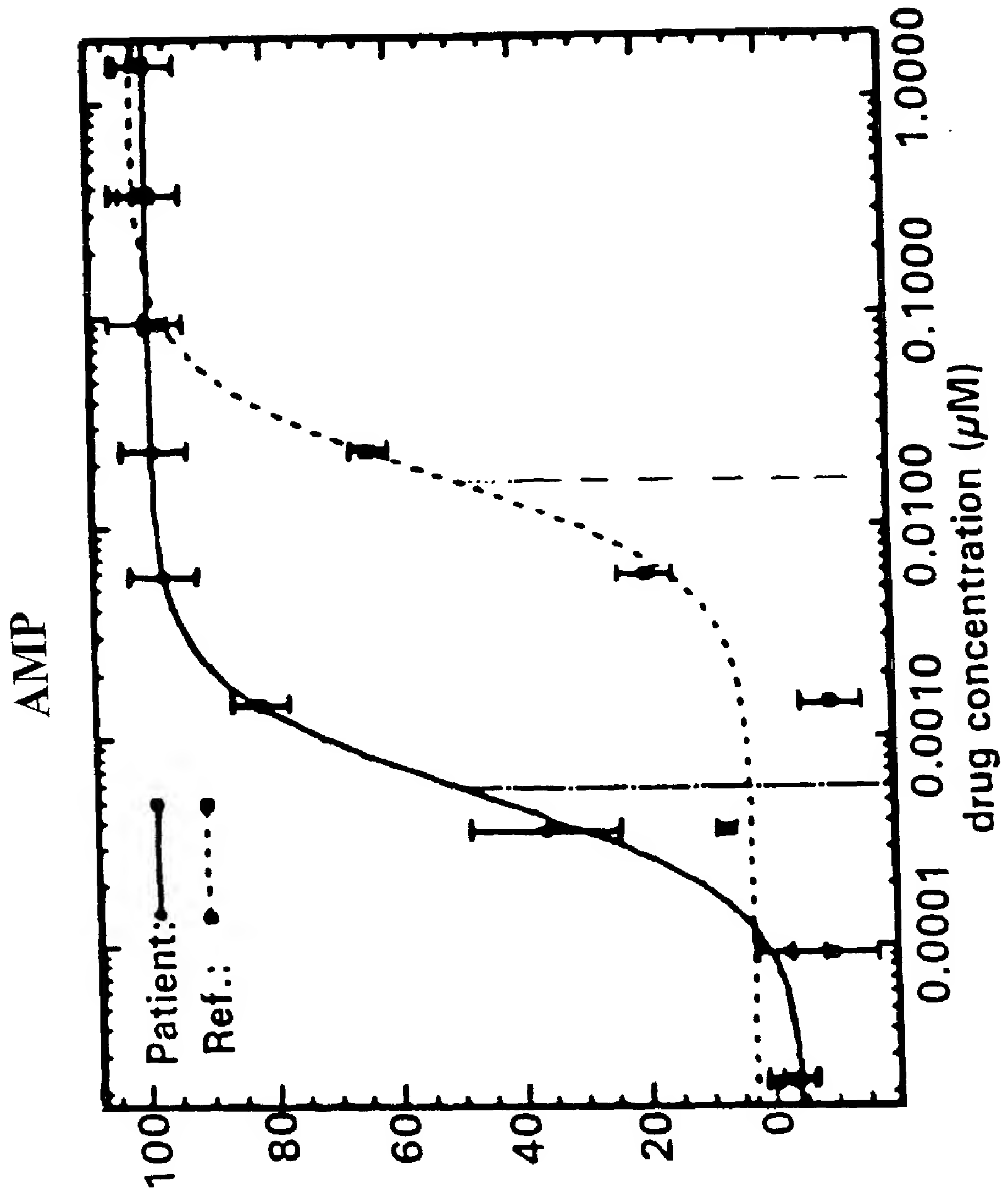
NFV





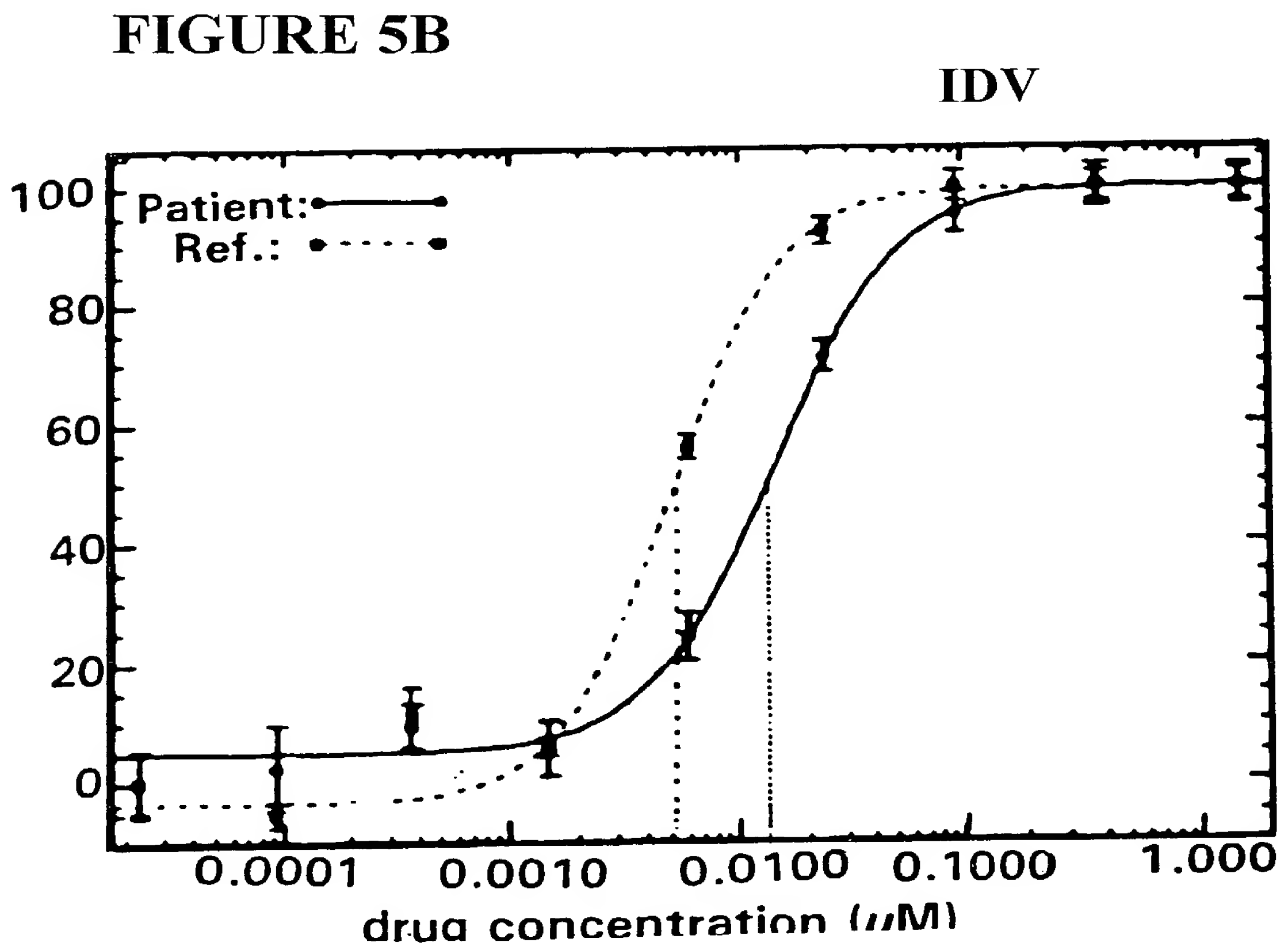
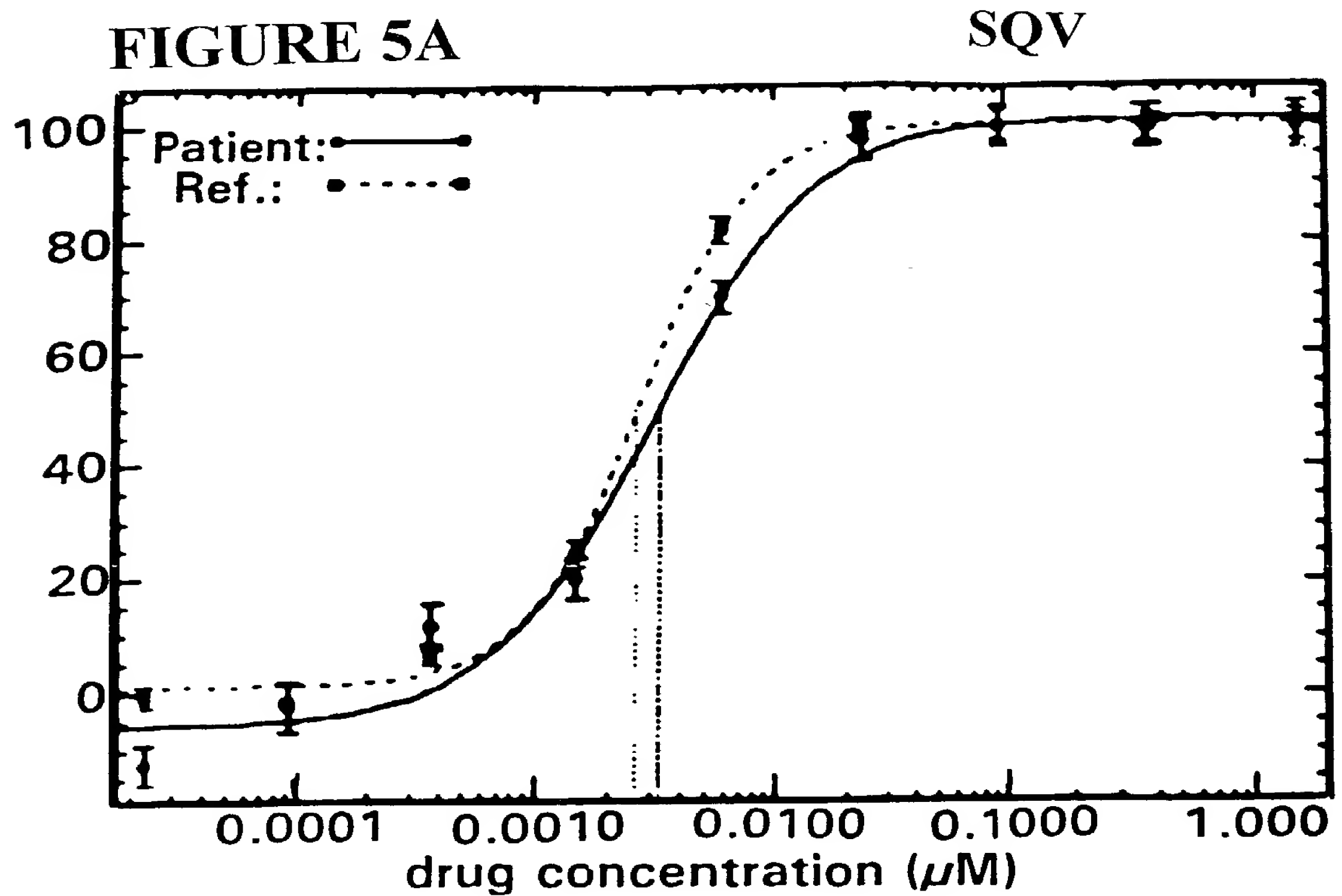
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FIGURE 4E





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FIGURE 5C

RTV

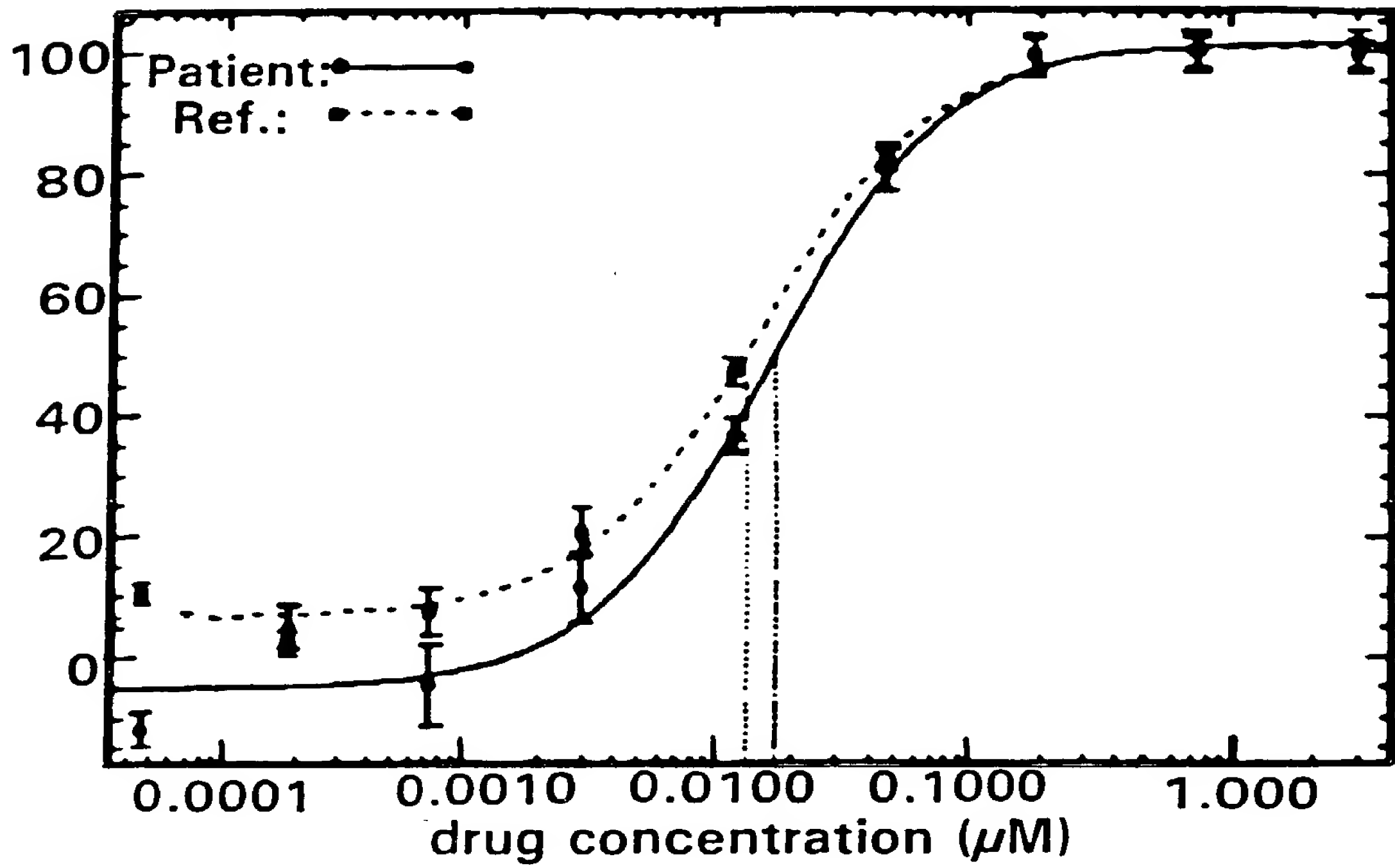
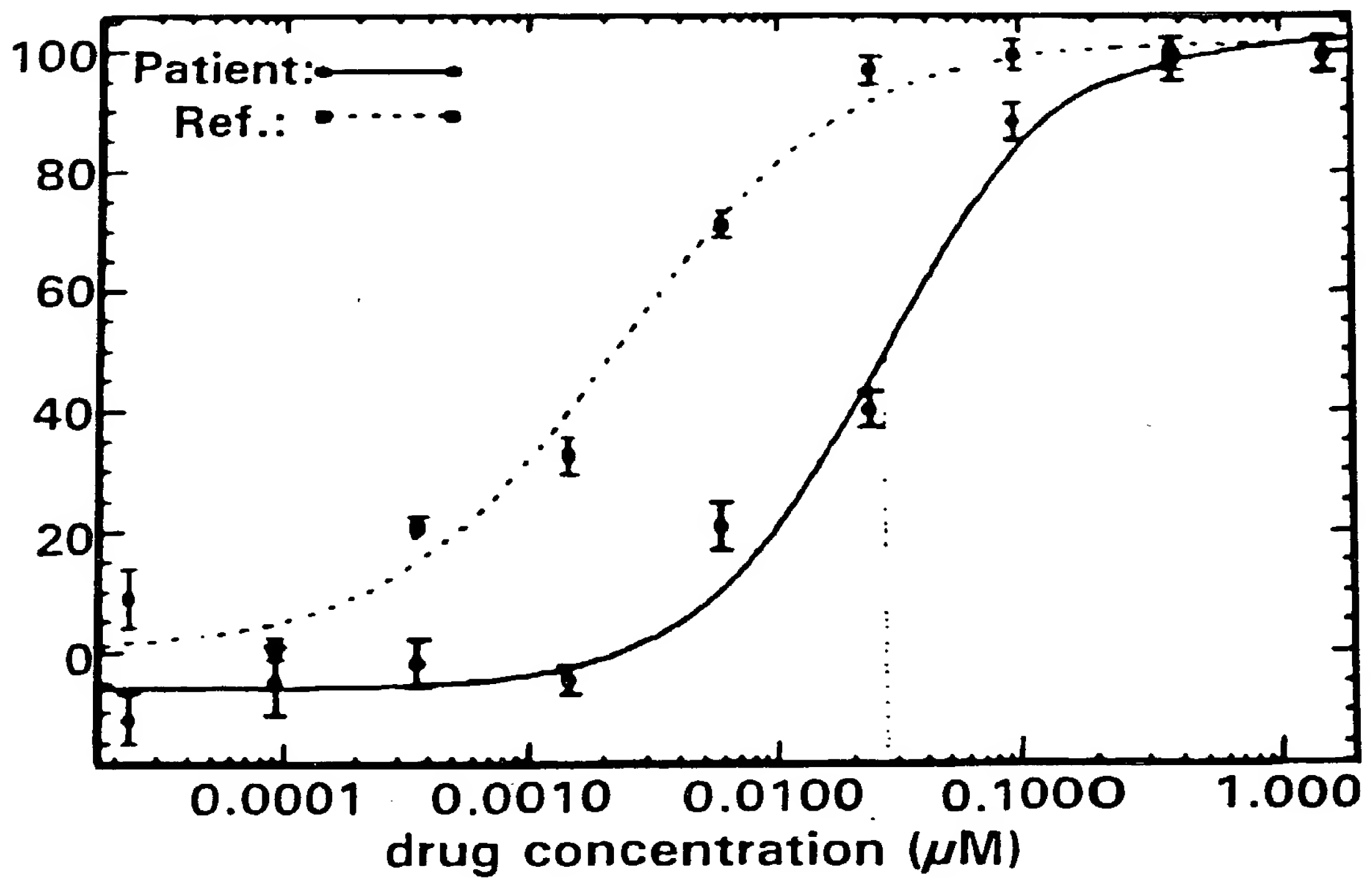


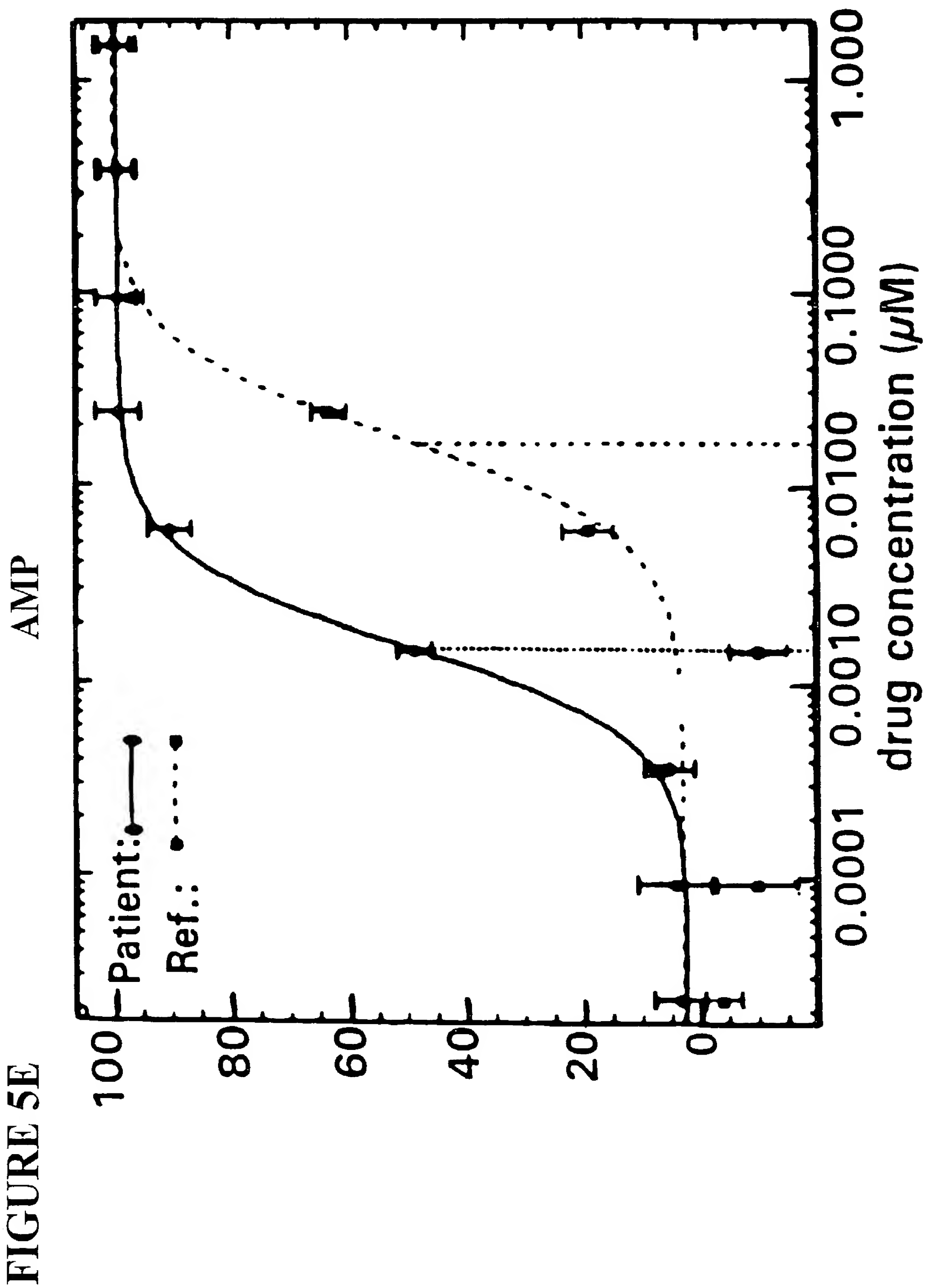
FIGURE 5D

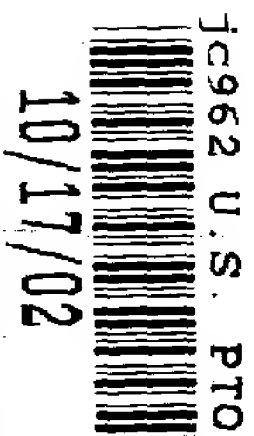
NFV





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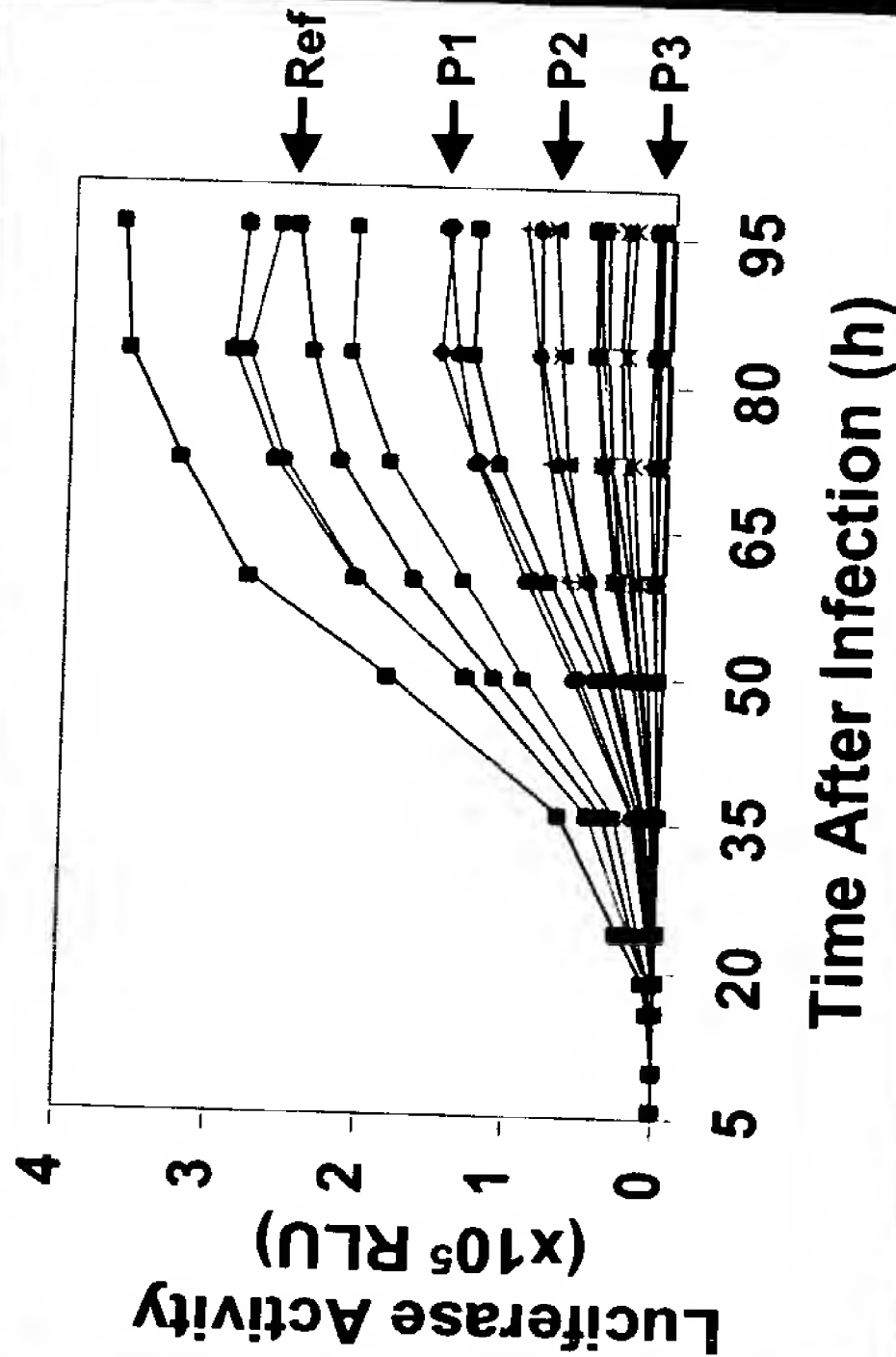




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FIGURE 6B

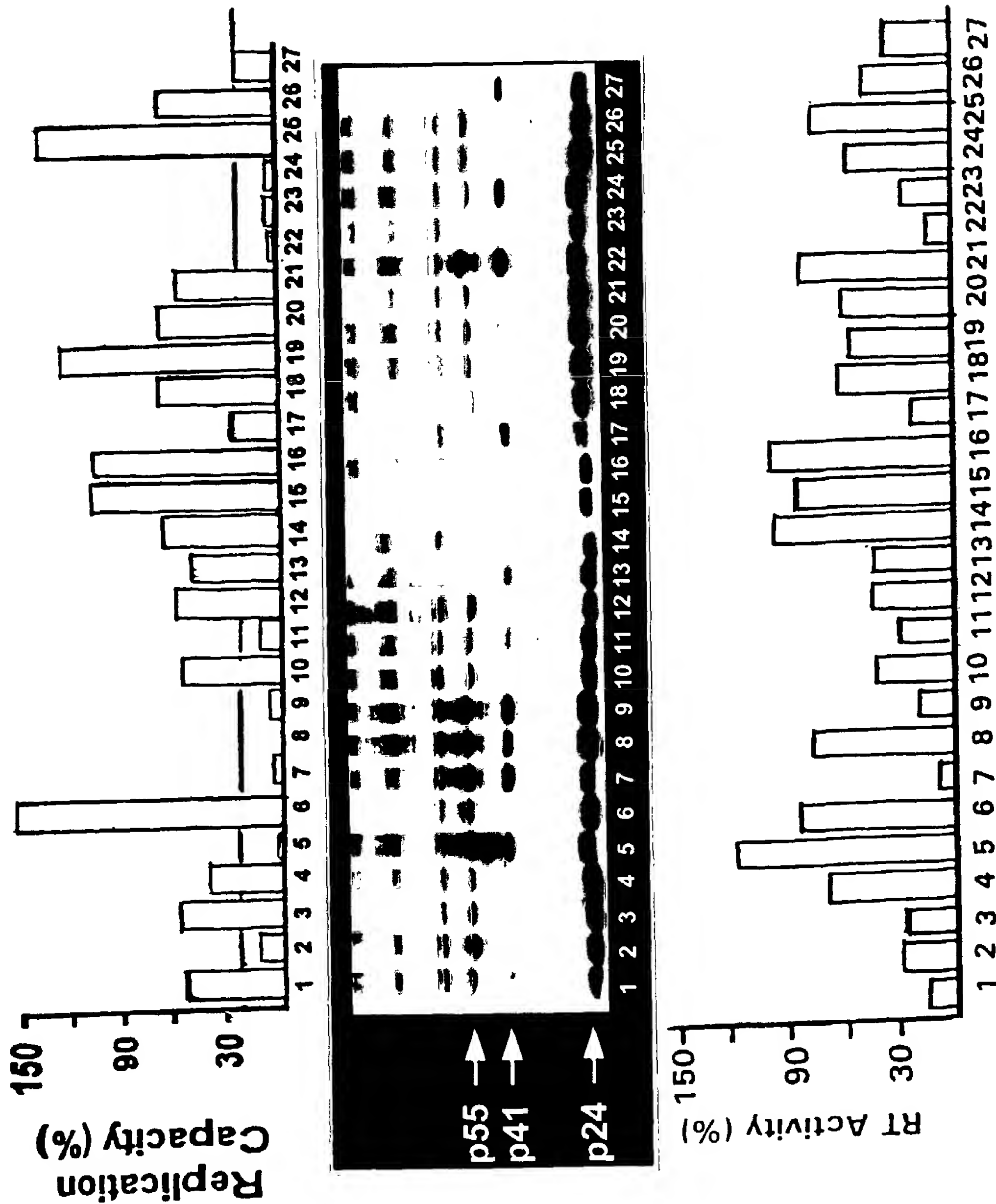
Figure B: Luciferase Activity in Infected Cells



Fold Resistance					
	P 1	P 2	P 3		
NRTI					
AZT	27	17	6		
3TC	>100	3	>100		
NNRTI					
NVP	40	0.3	0.3		
SQV	17	68	4		
IDV	30	47	39		
PRI					
RTV	11	62	63		
NFV	57	55	28		
AMP	4	18	3		

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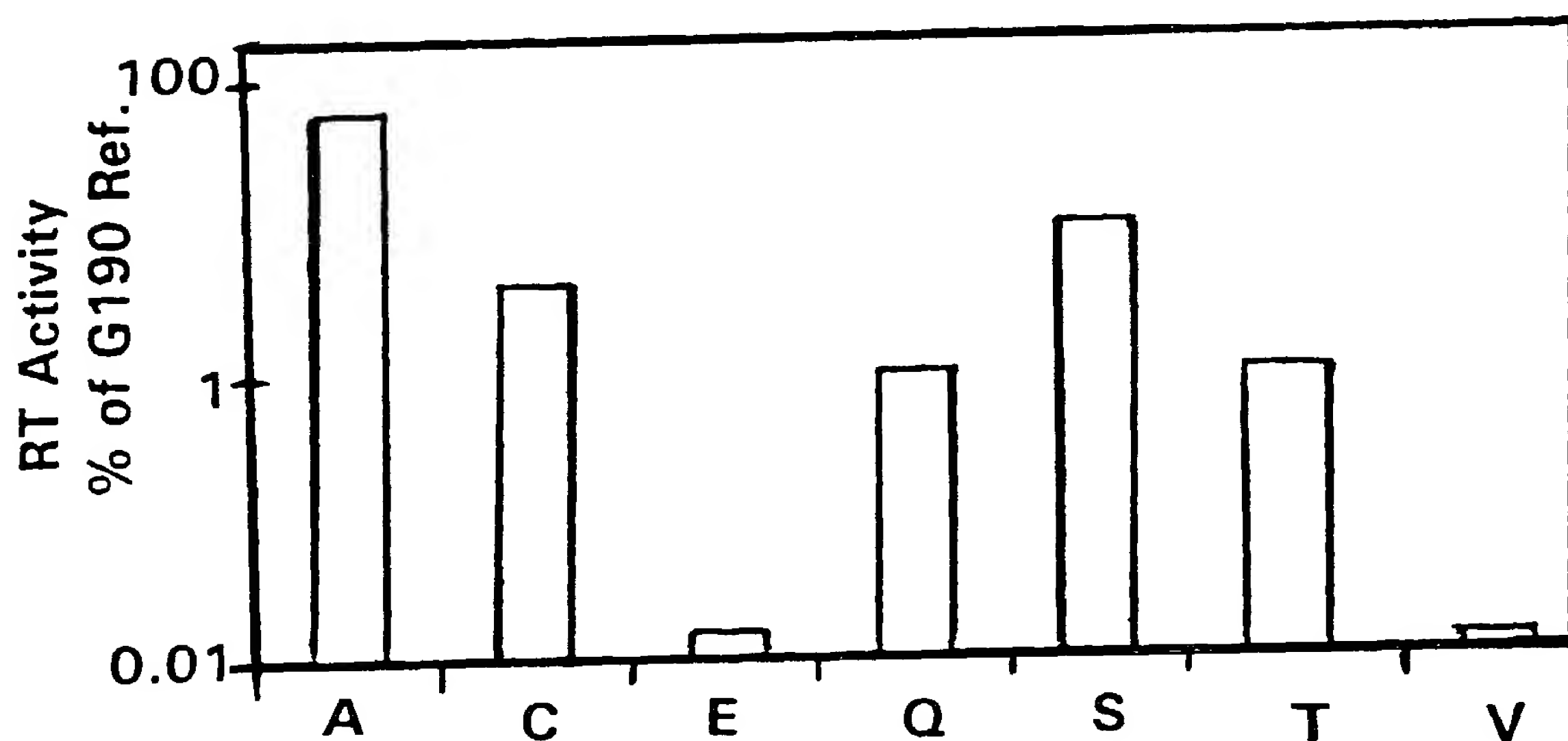
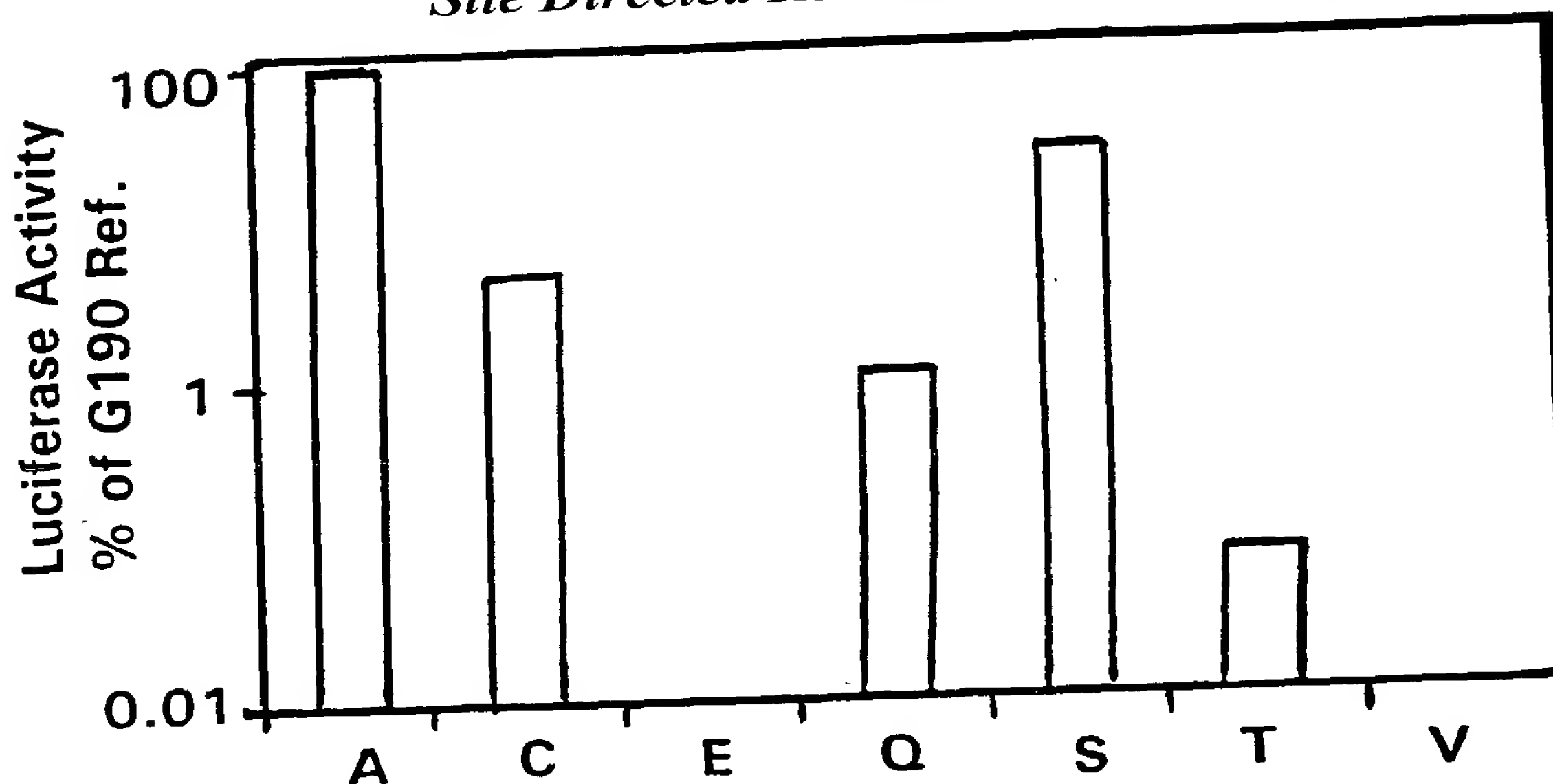
Replication Fitness, PR Processing, and RT Activity



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FIGURE 6D

Site Directed RT Mutants (G 190 Series)



G 190 Mutants

A = Ala	C = Cys
E = Glu	Q = Gln
S = Ser	T = Thr

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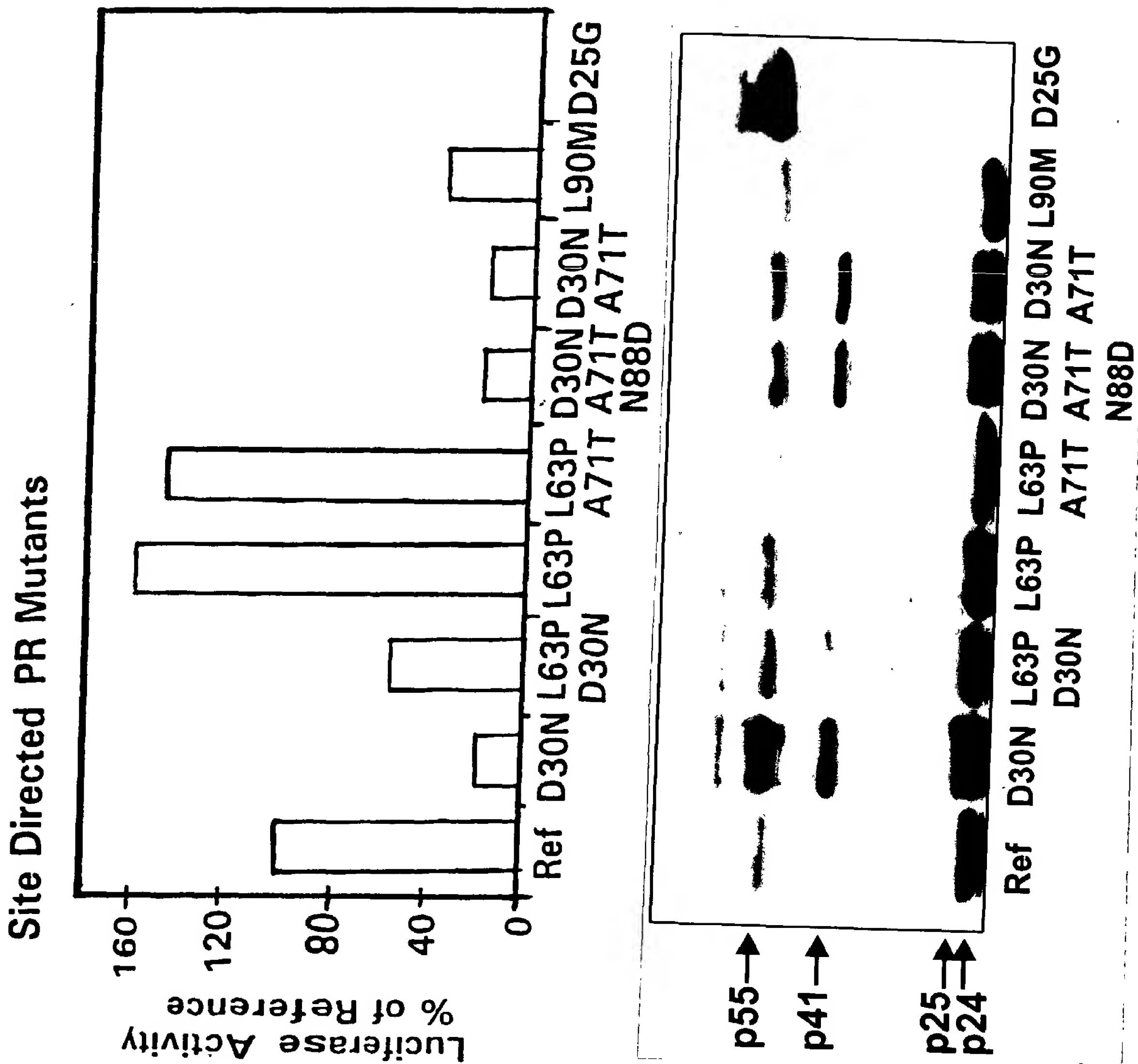
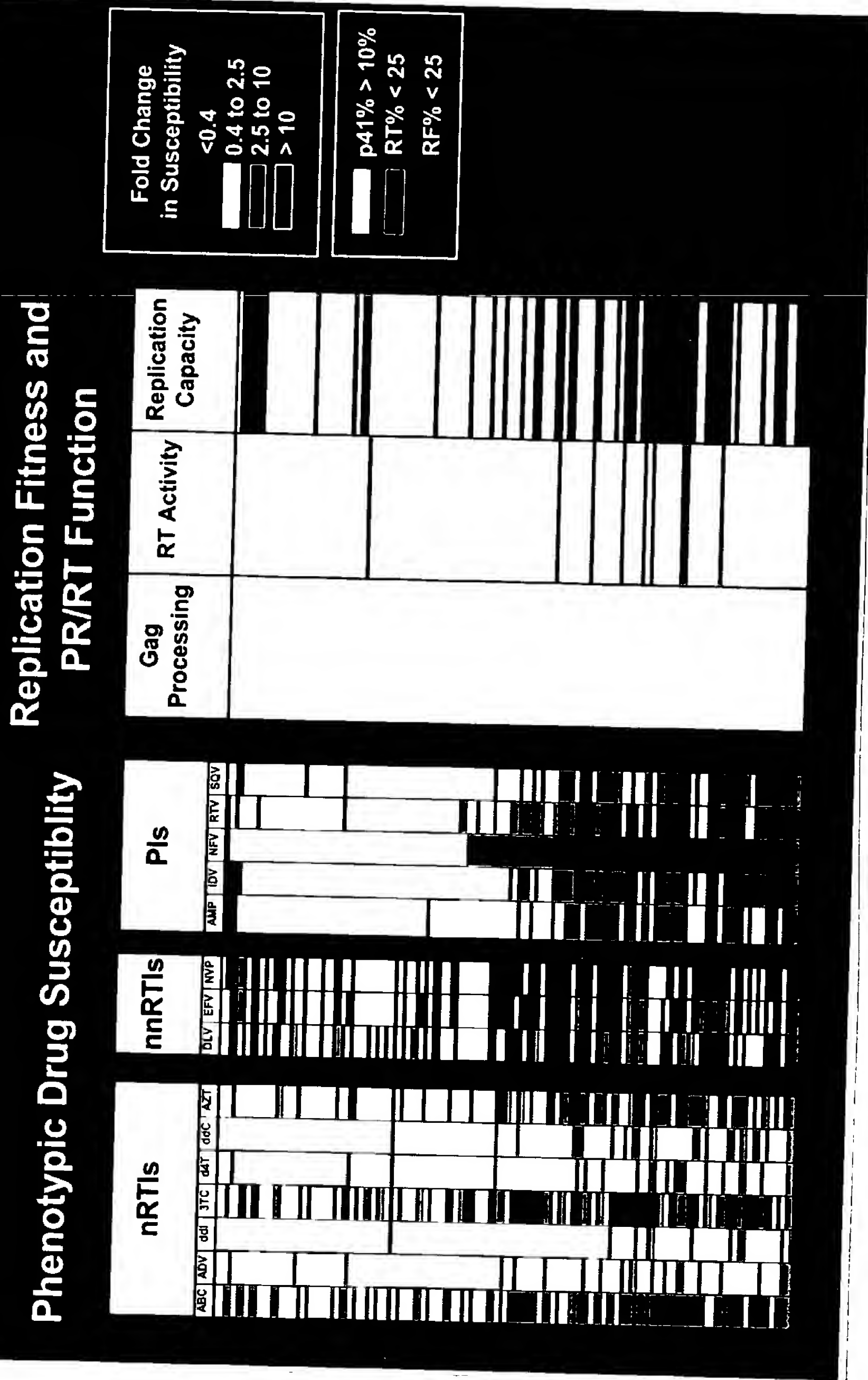


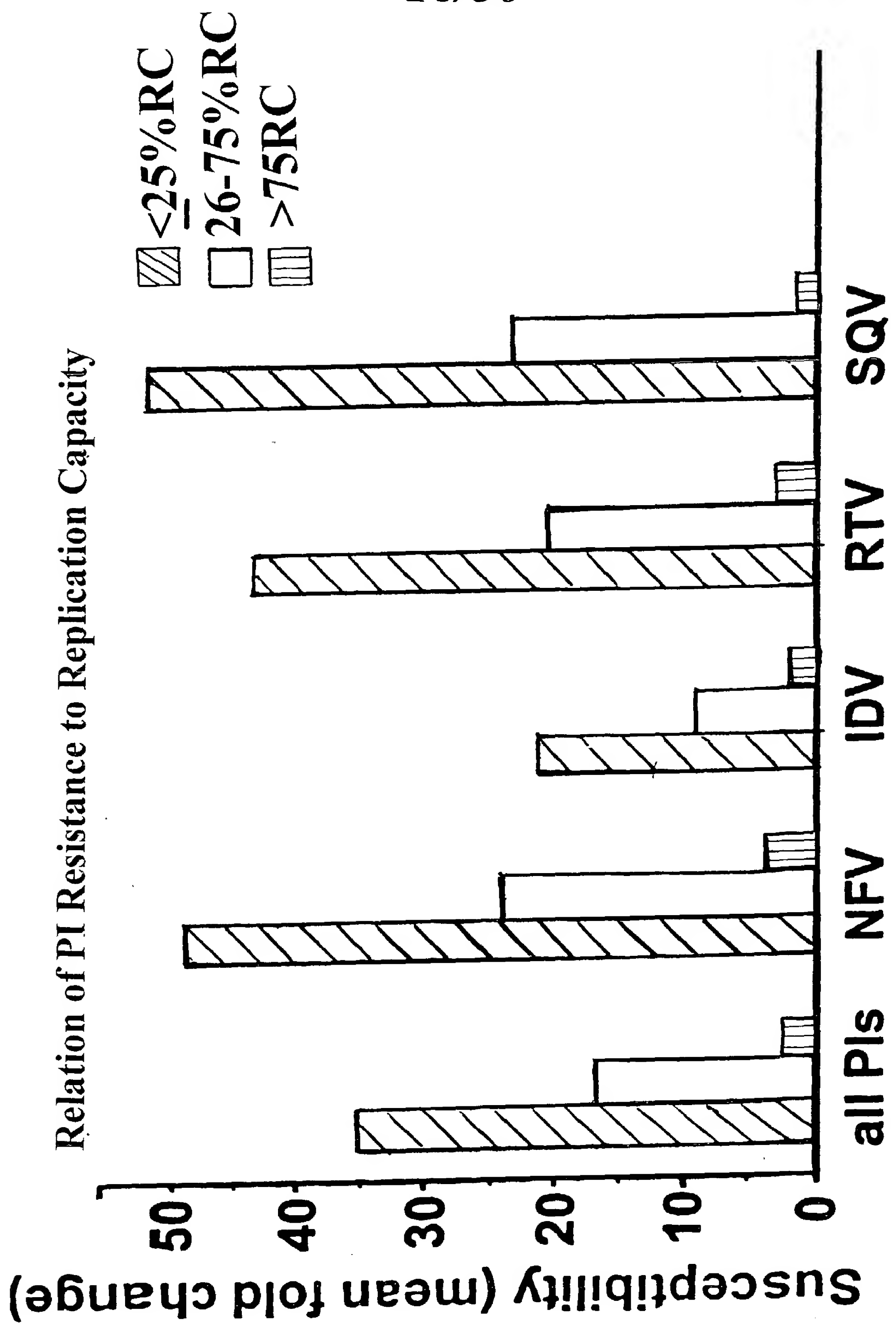
Figure F: Phenotypic Drug Susceptibility, Replication Fitness and PR/RT Function



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FIGURE 6G





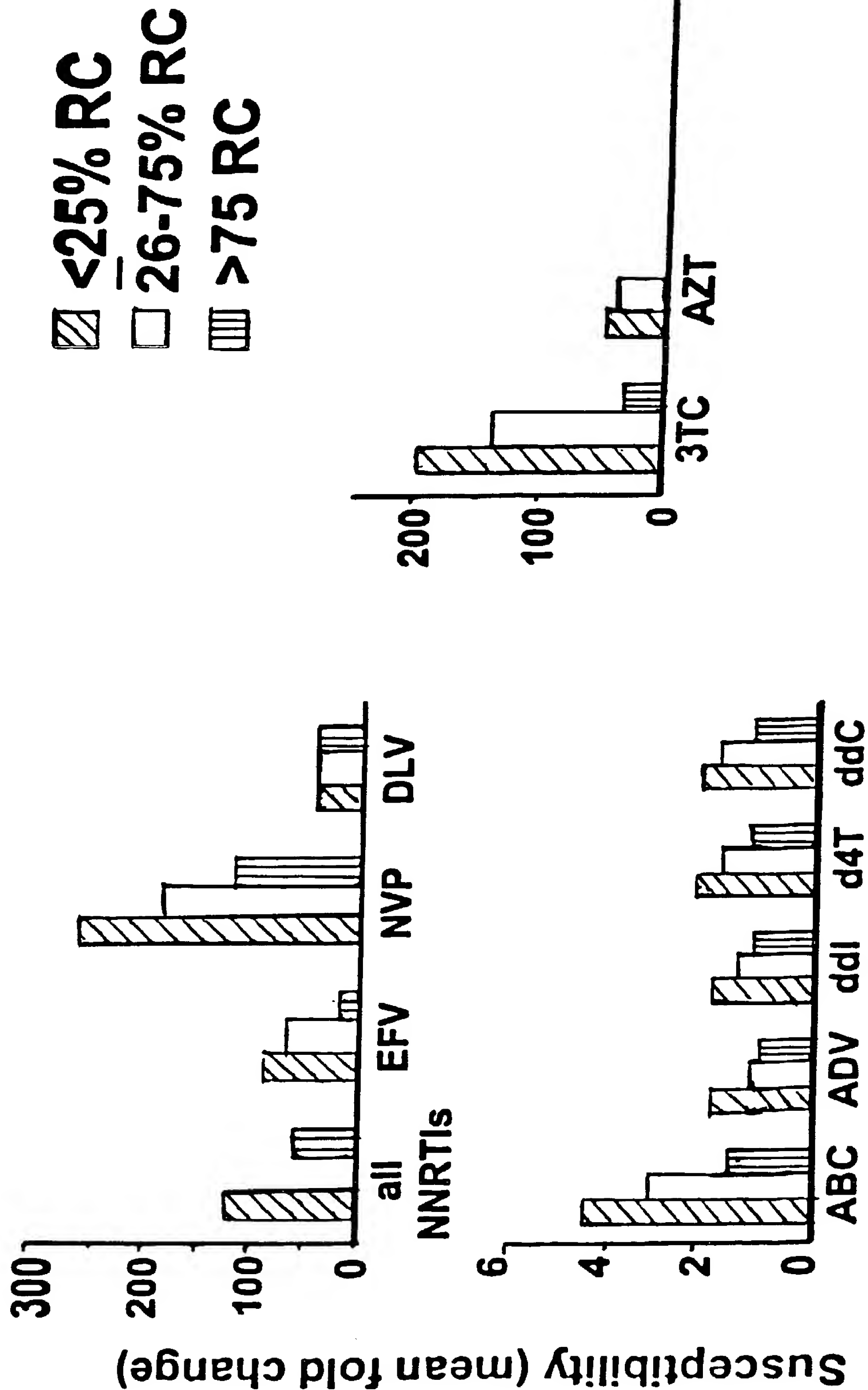
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THERAPEUTIC DECISIONS IN THE TREATMENT
OF HIV/AIDS

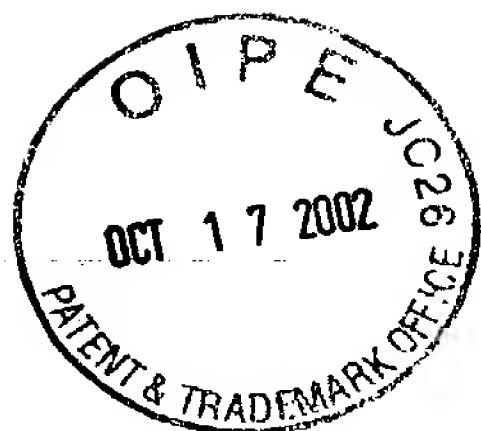
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FIGURE 6H

Relation of NRTI and NNRTI Resistance to Replication Capacity

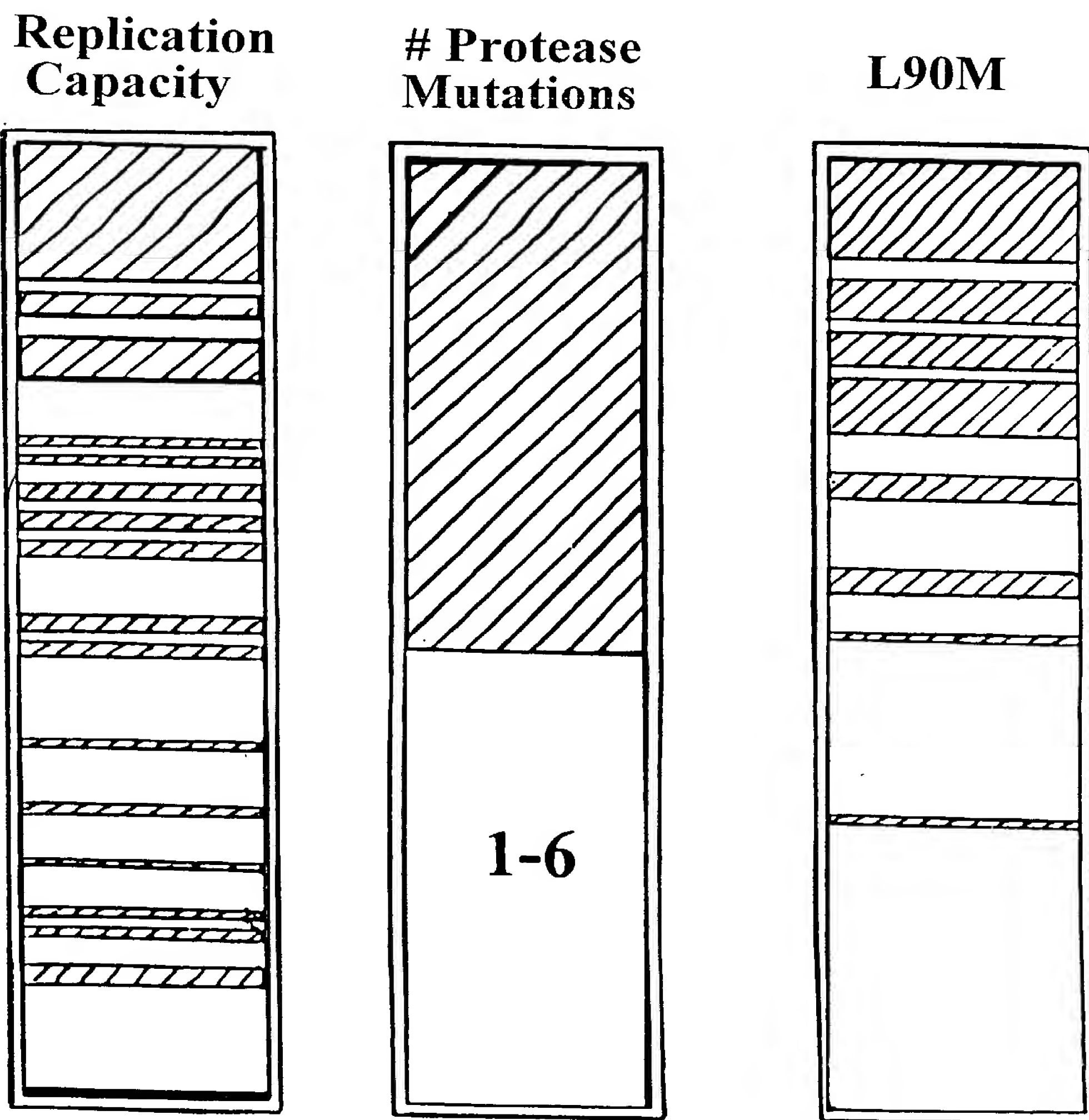




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FIGURE 6I

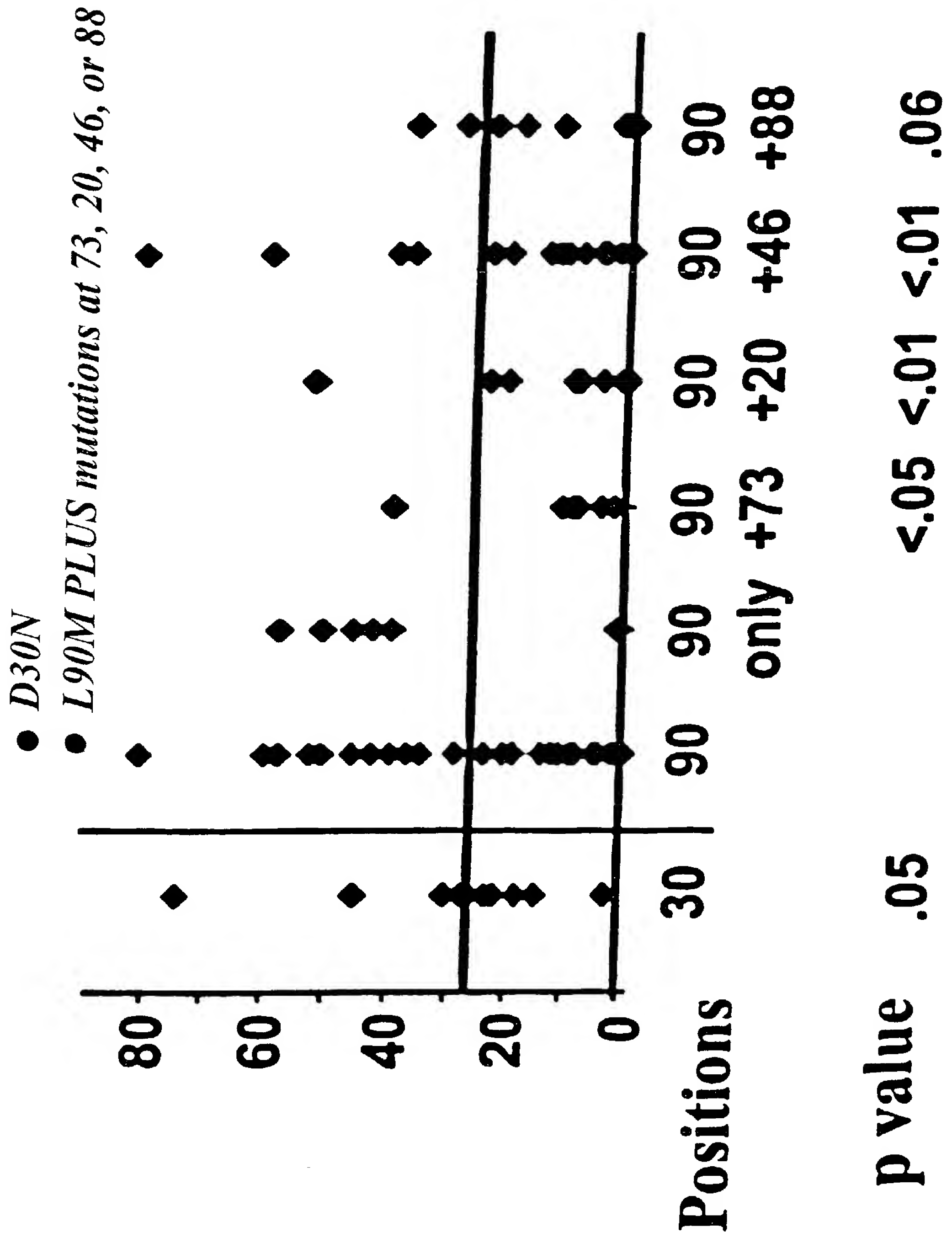
Low Replication Capacity is Associated with High
Numbers of Mutations in Protease and L90M





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FIGURE 6J
**Low Replication Capacity is Associated With Specific
Protease Mutations**

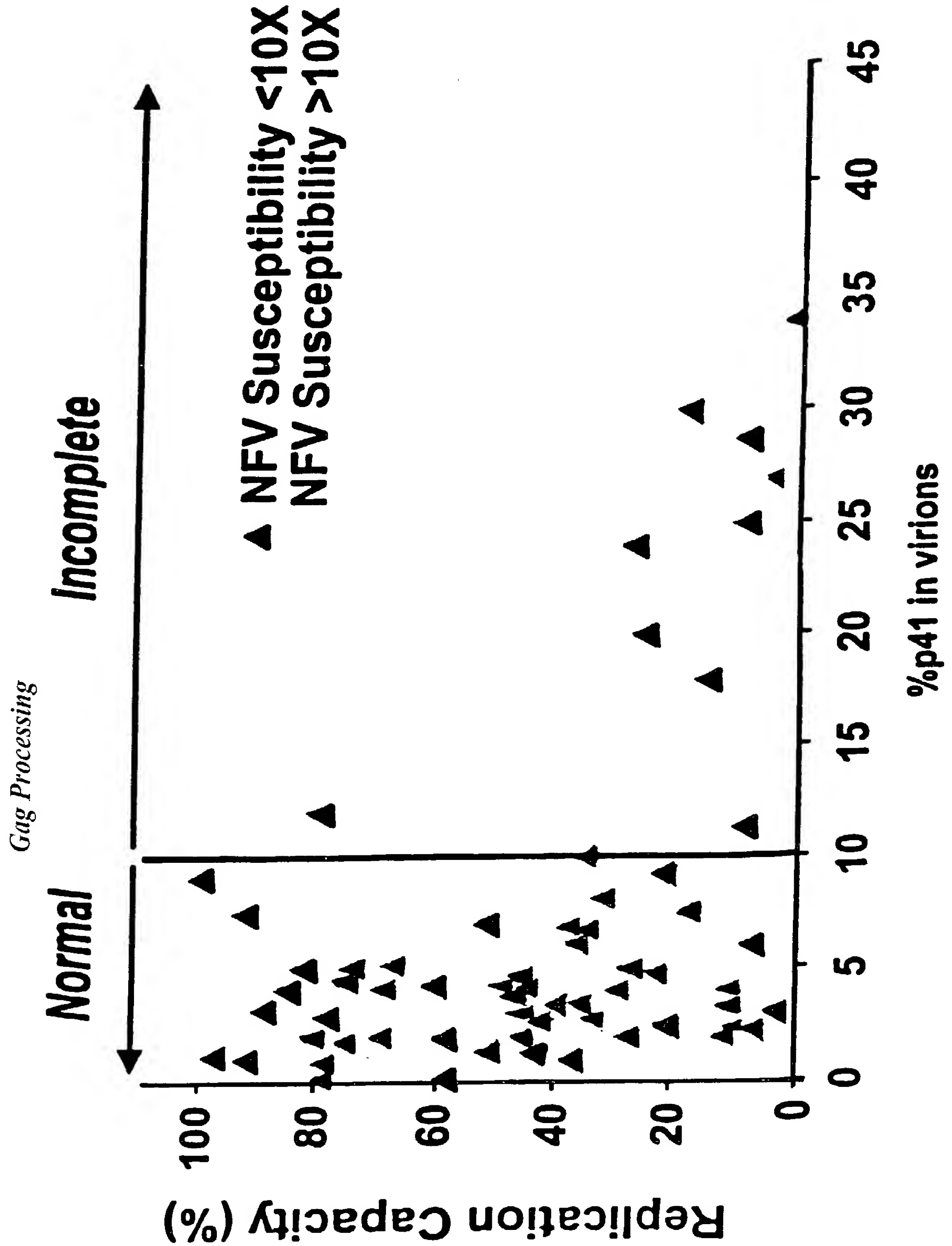




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Relation of NFV Phenotypic Drug Susceptibility, gag Processing and
Replication Fitness

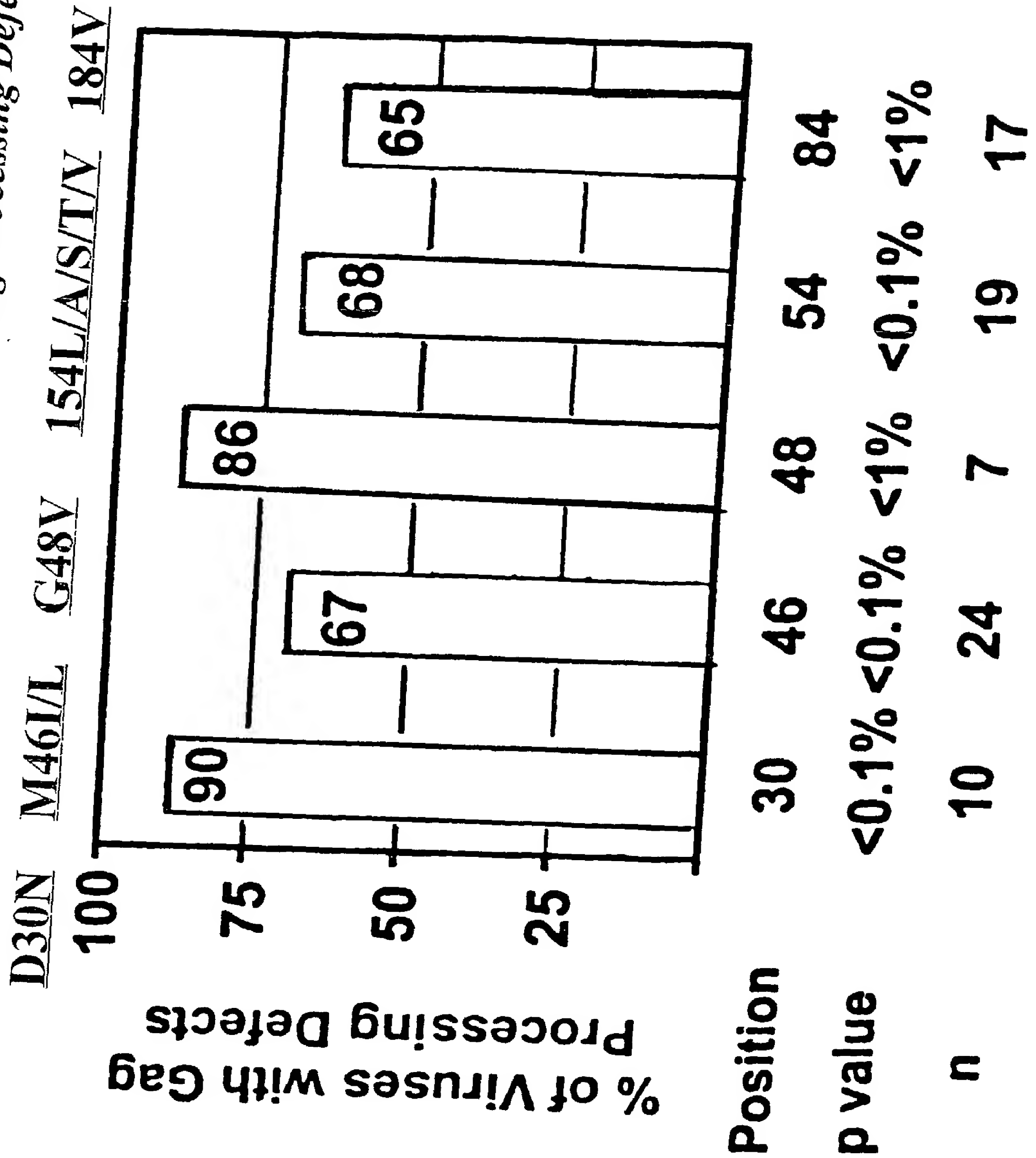
FIGURE 6K





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FIGURE 6L
Mutations in PR Associated with Gag Processing Defects





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FIGURE 6M

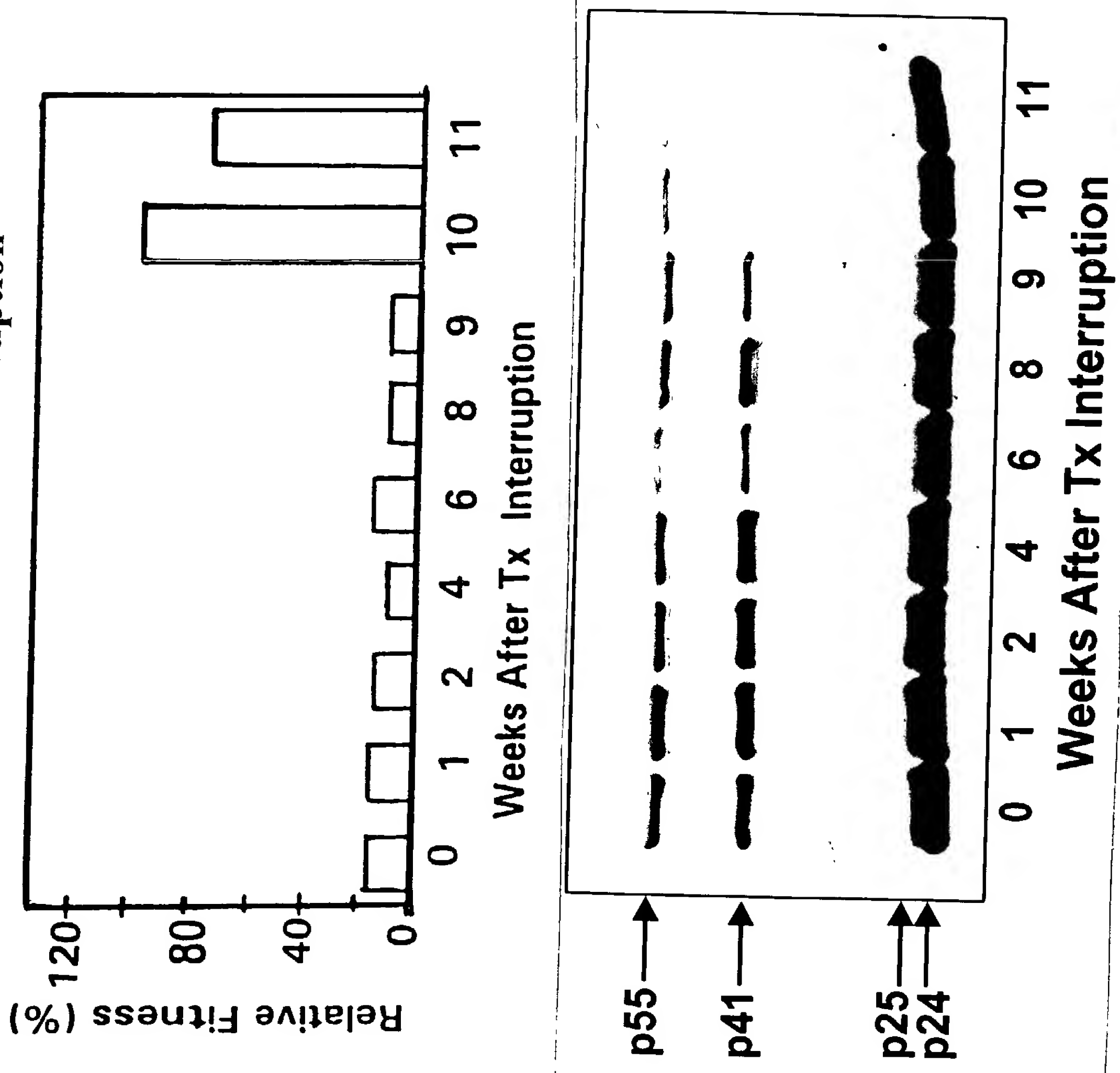
	NRTI					NNRTI				PI				
WEEK	AZT	3TC	D4T	ABC	NVP	DLV	EFV	SQV	IDV	RTV	NFV	AMP		
day 0	3.7	>100	2.8	19	>300	88	115	85	72	73	74	16		
1	4.5	>100	3.3	20	>300	78	134	95	74	59	80	21		
2	5.8	>100	3.2	14	>300	75	142	89	77	49	59	19		
3	6.5	>100	2.7	15	>300	96	183	59	75	52	51	15		
4	6.3	>100	3.1	15	>300	94	174	59	68	50	49	15		
5	6.4	>100	3.0	17	>300	76	119	59	60	54	36	10		
6	5.0	>100	2.8	19	>300	93	168	89	39	80	40	18		
7	9.1	>100	4.1	12	>300	89	154	85	78	53	53	19		
9	2.8	8.1	1.9	5.0	22	15	10	1.8	3.5	4.7	4.0	2.0		
10	1.5	1.7	1.1	1.3	1.7	2.0	1.6	0.9	1.6	1.9	1.8	1.6		
11	0.9	1.2	1.0	1.2	0.8	1.1	0.9	1.0	1.1	1.1	1.1	1.0		
12	0.8	1.3	0.8	1.2	0.5	1.0	0.8	0.8	0.8	0.9	1.1	0.8		
23	0.7	1.1	1.0	0.6	0.8	1.1	0.8	0.8	0.8	1.0	0.9	0.6		

Patient Virus Reversion to Drug Susceptibility After Treatment Interruption

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 Old S.N. 2960f

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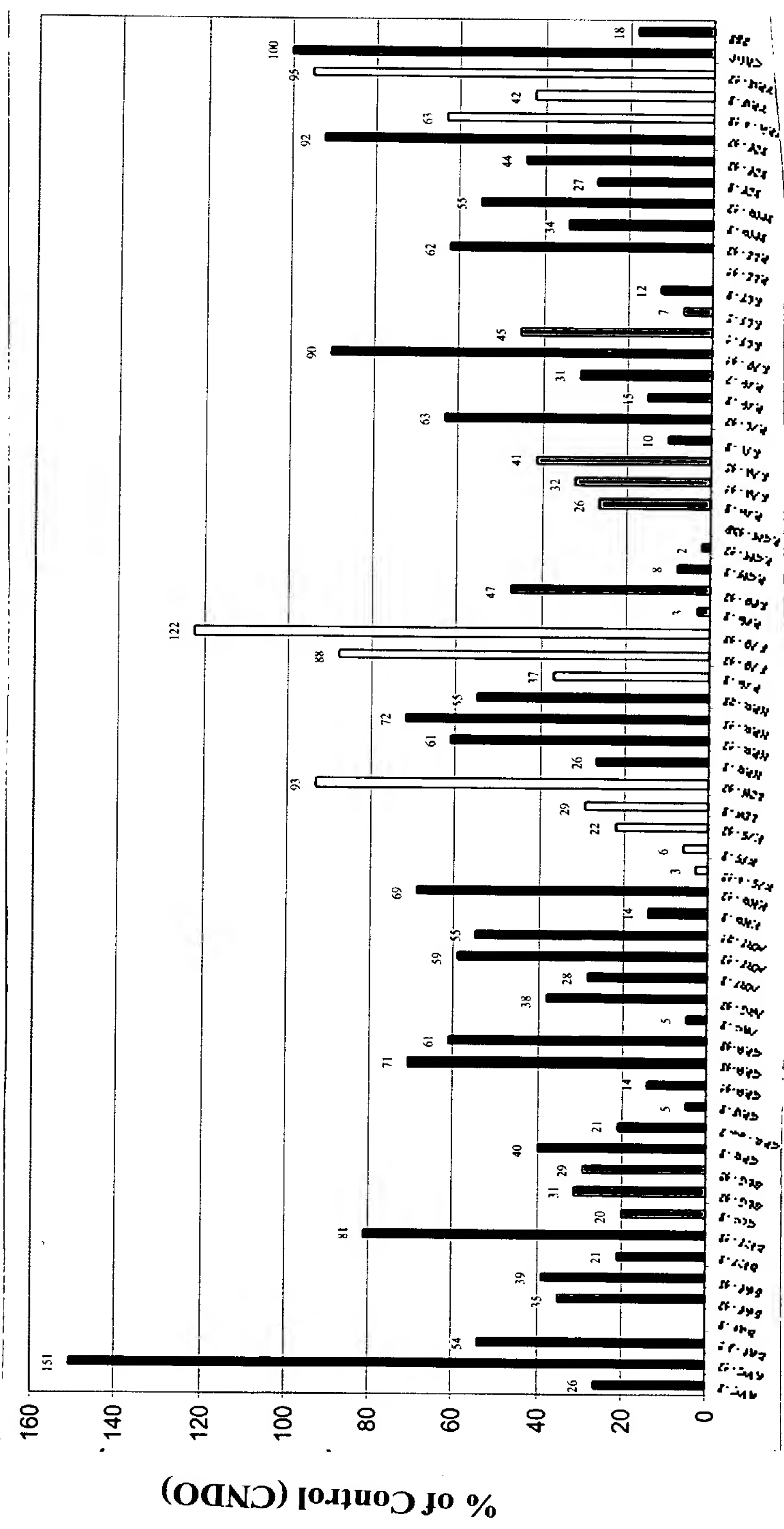
FIGURE 6N
 Patient Virus Reversion to Normal Replication Fitness after
 Treatment Interruption



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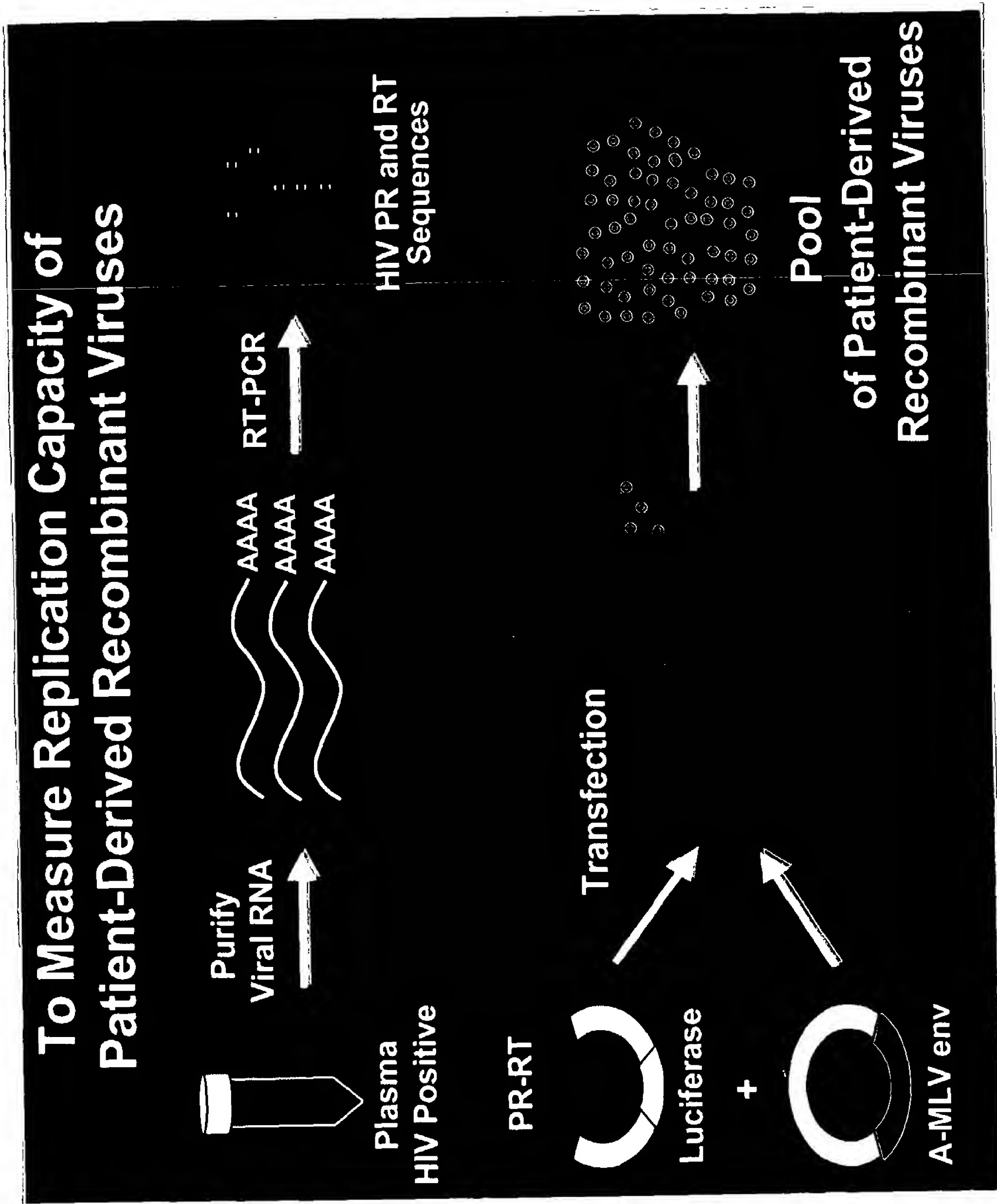
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FIGURE 60
Fitness on GCRC STI Samples (wk 0 and 12)-Assay#2
RLU corrected for p24 input (% of control)



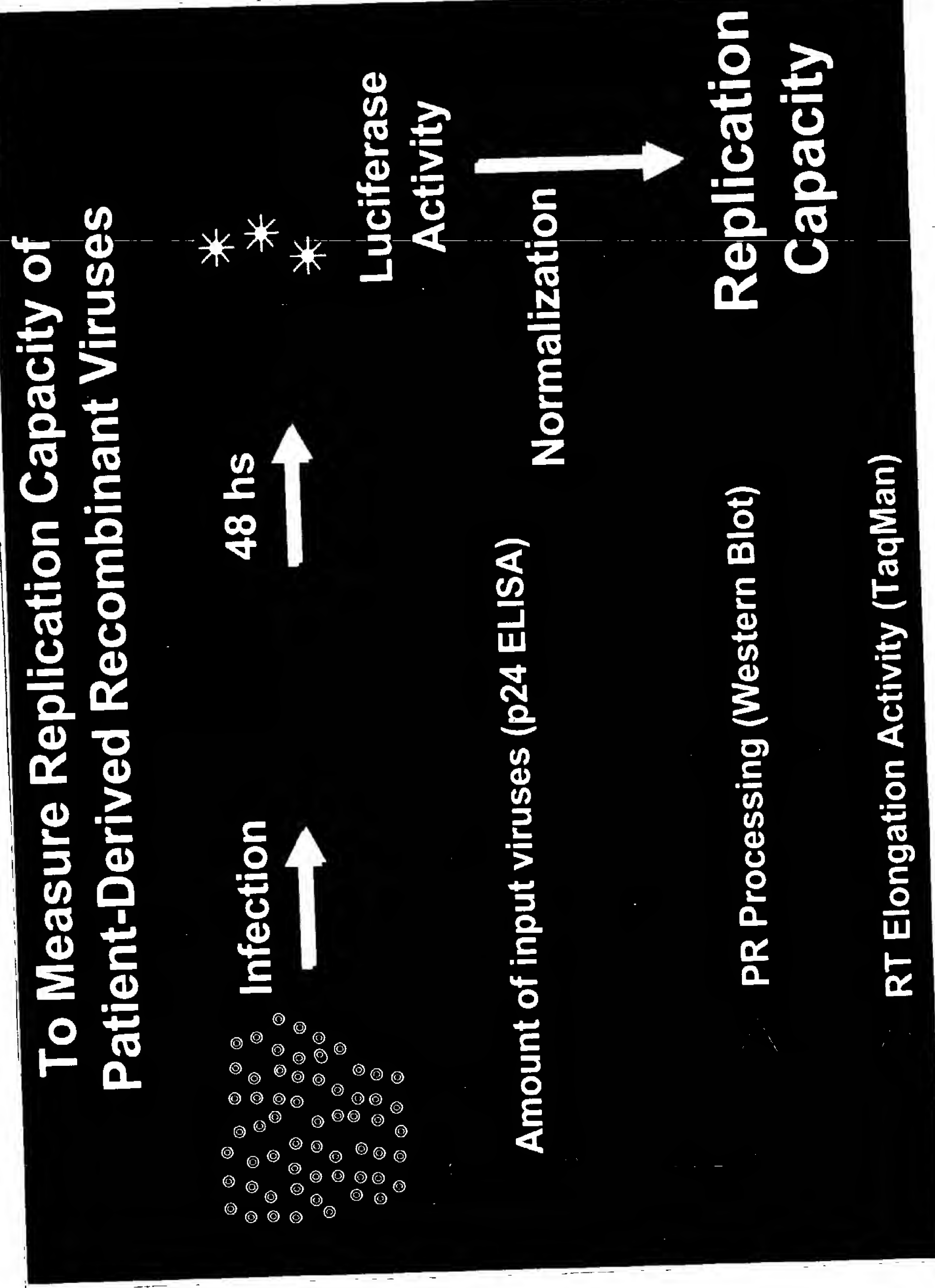
Patient post STI

FIGURE 6P



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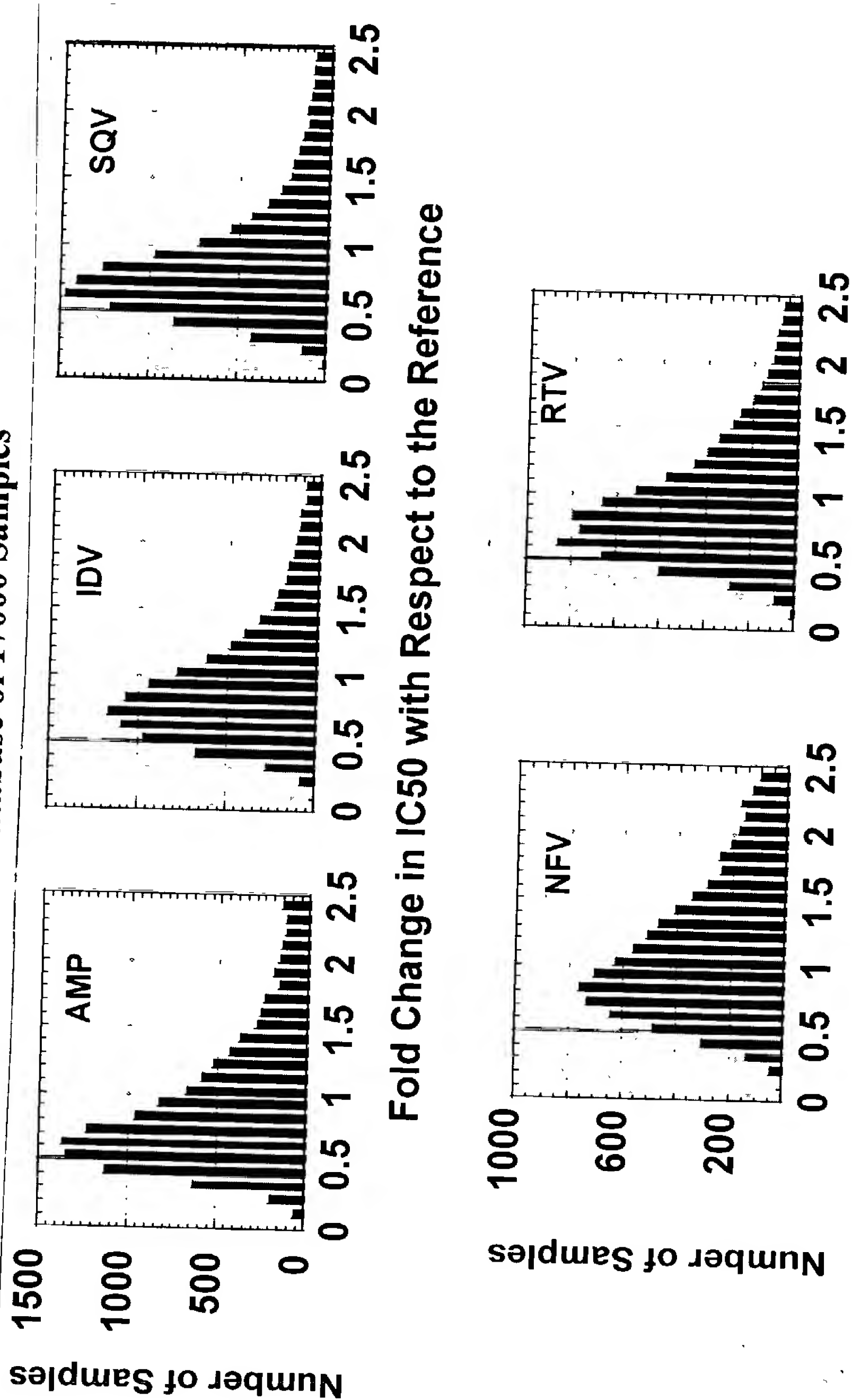
FIGURE 6Q



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09074472 101702

FIGURE 6



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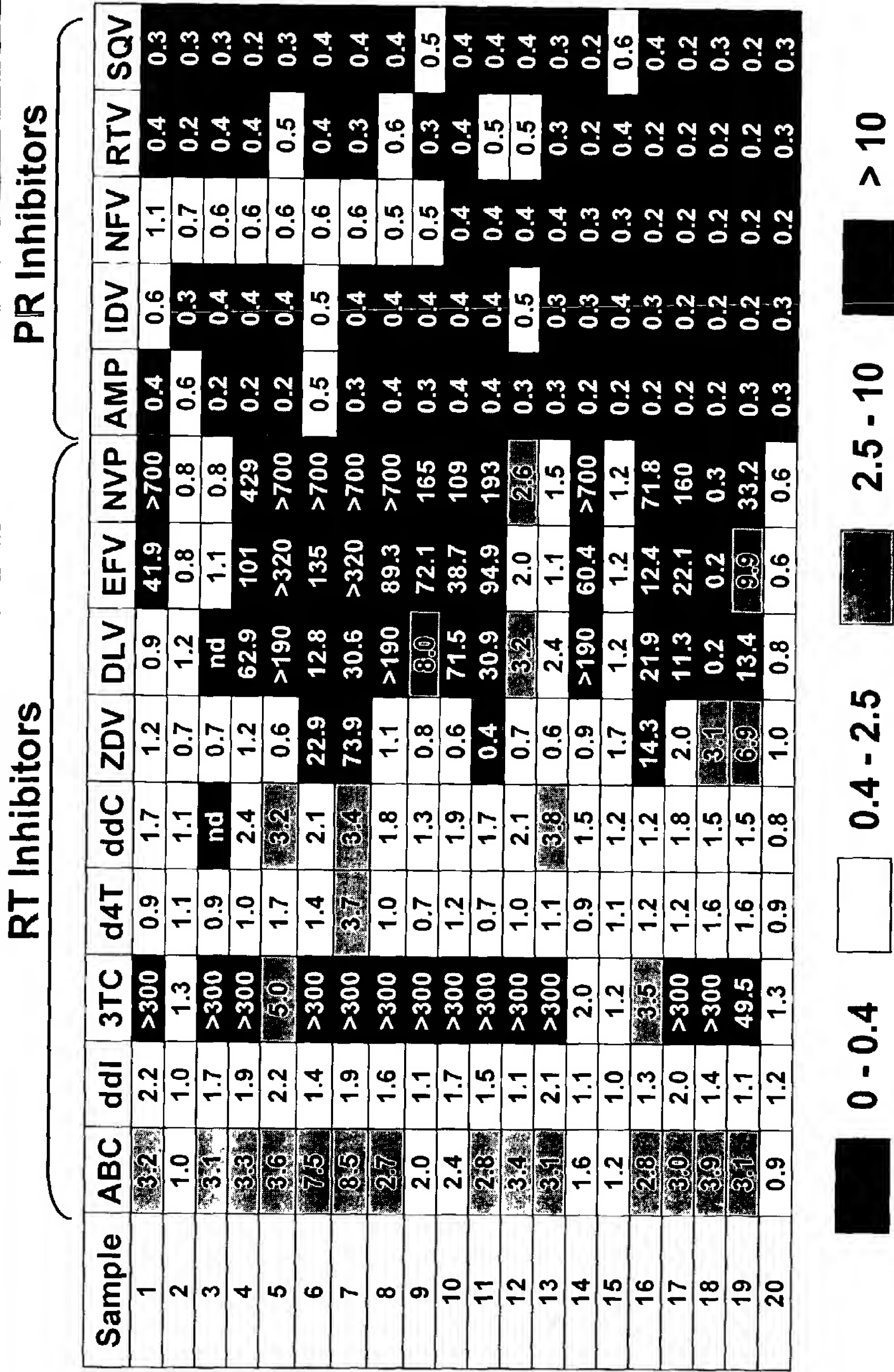
OLD S n 29601

09/07/01 10:17:02

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FIGURE 7

Fold Change Susceptibility
20 Randomly Selected Patient Viruses with HS to PIs



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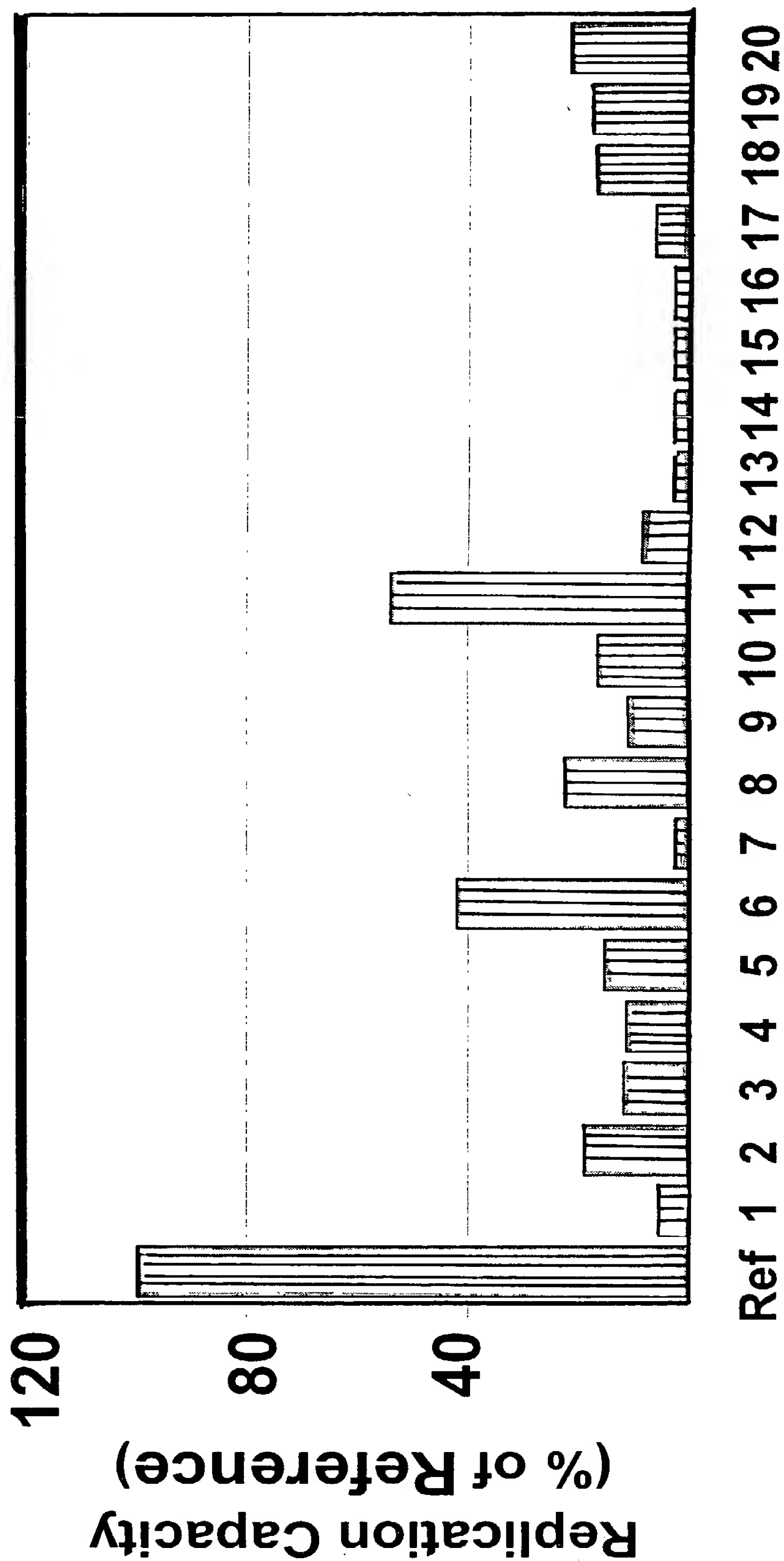


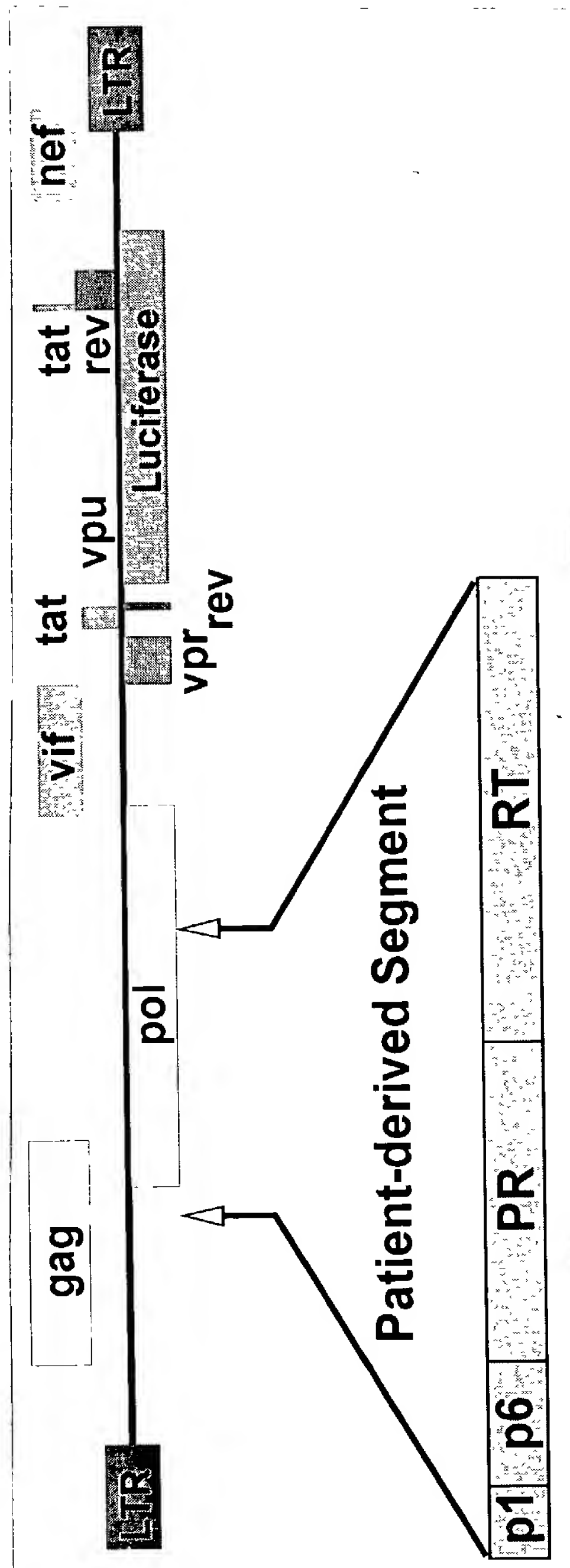
FIGURE 8

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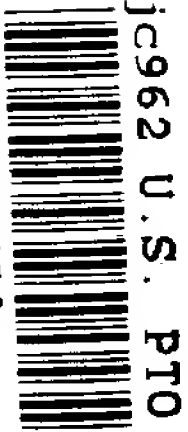
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FIGURE 9

*Cell based assay to measure phenotypic drug susceptibility employing
 patient-derived recombinant viruses*



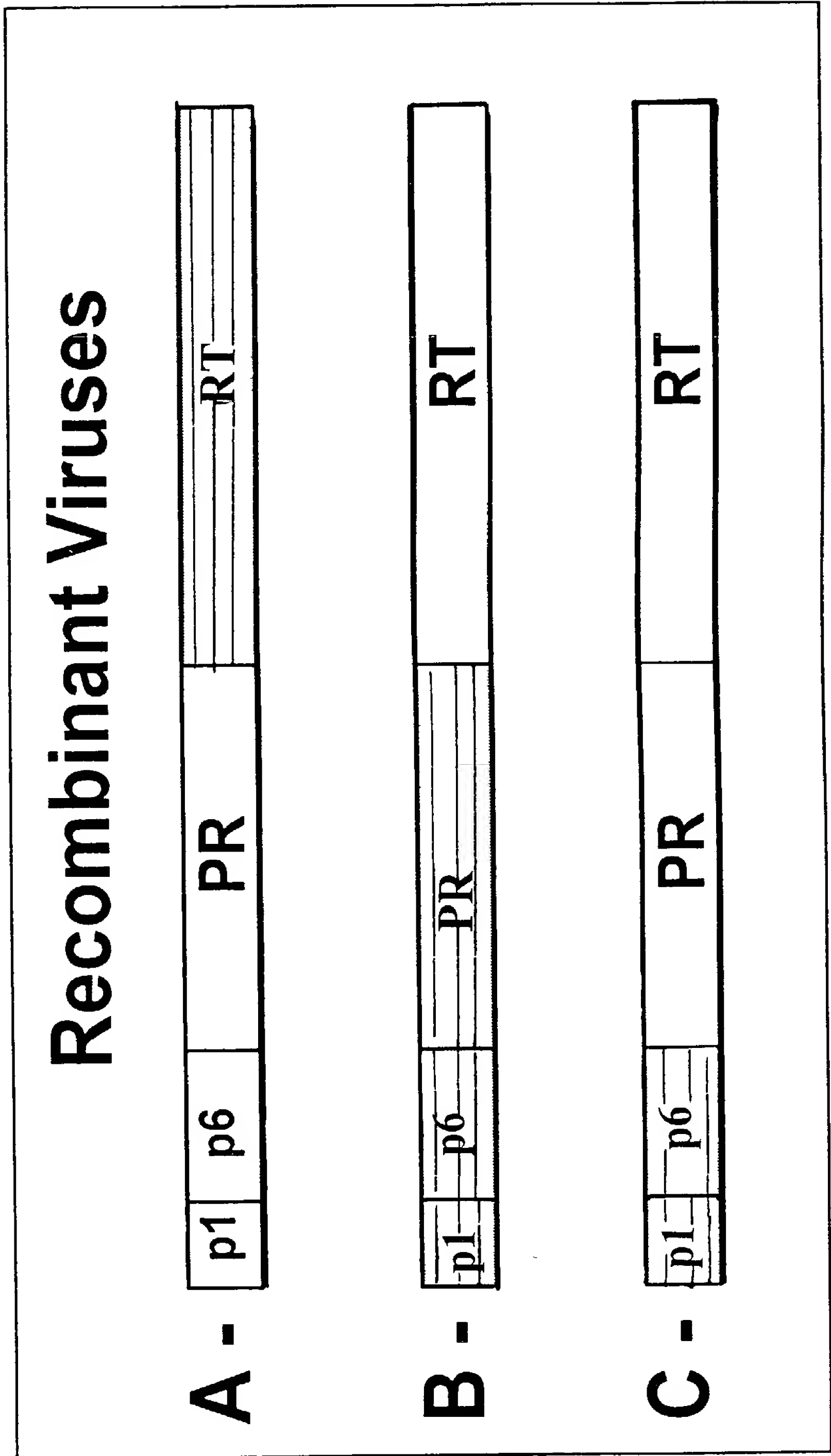
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FIGURE 10



 NL4-3 Sequence
 Patient Sequence

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(Musical notation continues)

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FIGURE 11

A -

p1	p6	PR	RT
----	----	----	----

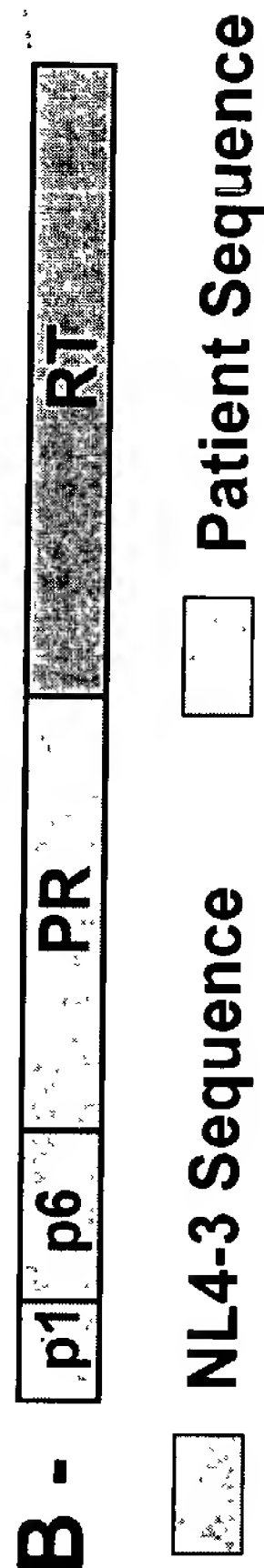
Fold Change in Susceptibility

Sample	ABC	ddl	3TC	d4T	ddC	ZDV	DLV	EFV	NVP	AMP	IDV	NFV	RTV	SQV
1	2.5	1.5	>300	0.8	1.5	0.8	0.7	35.8	>700	0.7	1.0	1.1	0.9	0.9
2	1.0	1.2	1.4	1.0	1.1	0.7	1.5	0.8	0.8	0.7	0.8	1.0	0.9	0.8
3	4.4	1.8	>300	0.9	2.1	0.7	2.1	1.1	1.4	0.6	0.9	0.9	0.7	0.4
4	3.5	1.8	>300	0.9	1.8	1.1	85.9	141	344	0.6	0.8	0.9	0.8	0.8
5	2.7	2.1	8.9	1.4	3.1	0.5	>190	>320	>700	0.5	1.0	1.1	0.7	1.0
6	7.0	1.4	>300	1.5	2.6	9.8	5.8	189	>700	0.7	0.5	0.8	0.7	0.7
7	9.9	2.6	>300	3.3	3.0	80.1	48.1	>320	>700	0.7	0.8	0.9	0.8	0.5
8														
9	1.9	1.1	>300	1.2	1.1	1.1	31.4	170	>700	0.7	0.7	1.4	0.8	0.9
10	3.8	1.8	>300	0.9	2.3	0.8	73.3	50	100	0.7	0.8	1.0	0.8	1.0
11	2.3	1.5	>300	0.7	1.7	0.5	35.6	130	182	0.6	1.1	1.0	1.0	0.8
12	4.3	1.9	>300	0.9	2.3	0.8	2.2	1.2	1.5	0.9	0.9	1.2	1.0	1.0
13	3.4	1.6	>300	1.0	2.1	0.4	2.1	0.8	1.2	0.8	1.0	1.0	1.0	1.0
14	5.7	1.8	>300	1.8	2.2	7.7	0.5	0.6	0.7	0.5	0.5	0.7	0.8	0.7
15	1.6	1.1	1.0	1.0	1.0	1.6	1.1	1.2	1.2	0.8	1.1	1.2	1.0	1.1
16	3.3	1.3	4.0	1.4	1.3	31	47.9	25	106	0.5	0.5	0.8	0.6	0.7
17	3.9	1.6	>300	0.8	2.0	2.2	12.6	33	166	0.5	0.8	0.7	0.9	0.7
18	5.7	1.8	>300	1.8	2.2	8	0.5	0.6	0.7	0.5	0.5	0.7	0.8	0.7
19	4.4	1.6	79.1	1.3	1.8	20	29	24	78	0.3	0.6	0.6	0.5	0.7
20	1.0	1.1	1.0	1.1	1.1	0.8	1.1	0.6	0.6	1.0	1.1	1.2	1.1	1.2

0 - 0.4	0.4 - 2.5	2.5 - 10	> 10
			

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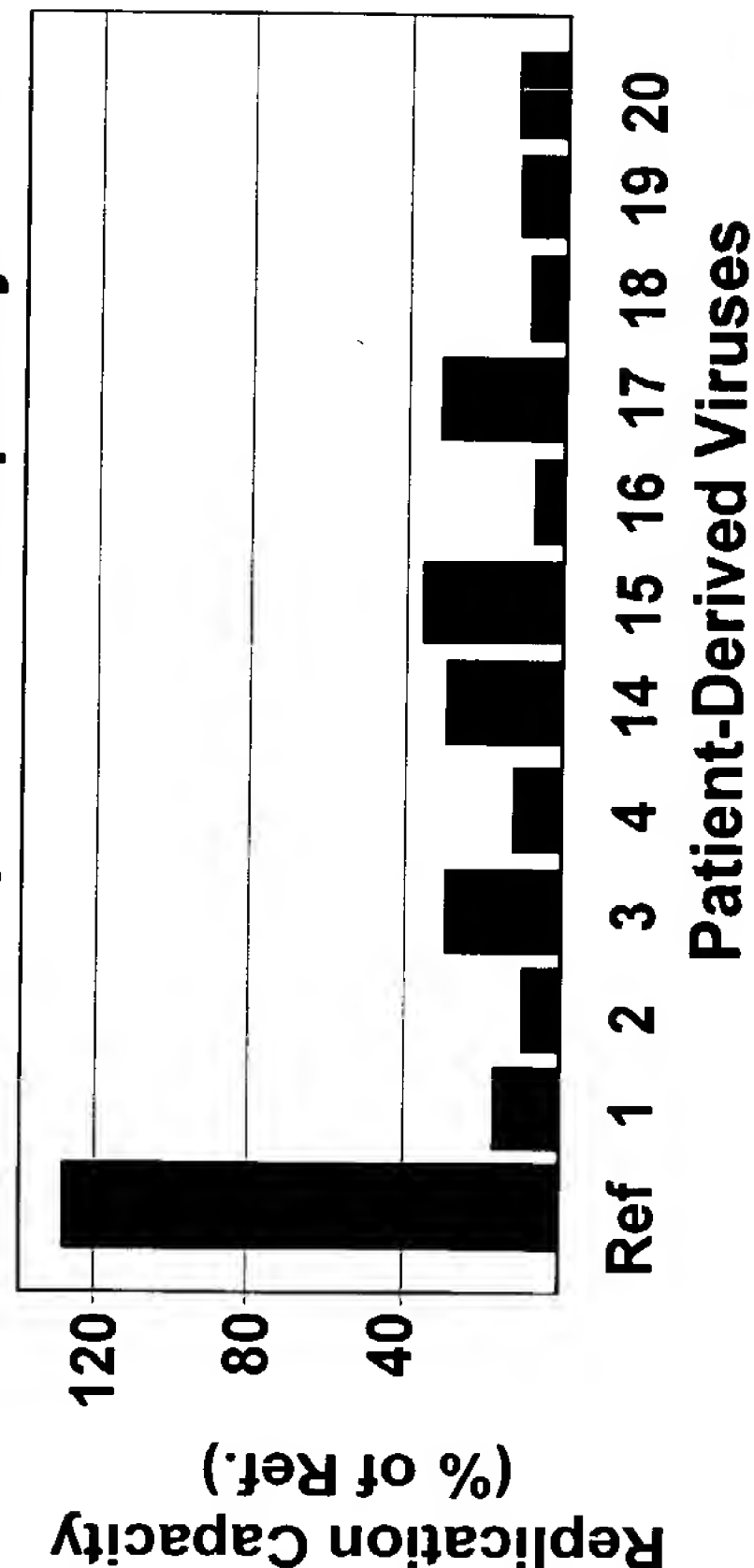
FIGURE 12



Fold Change in Susceptibility

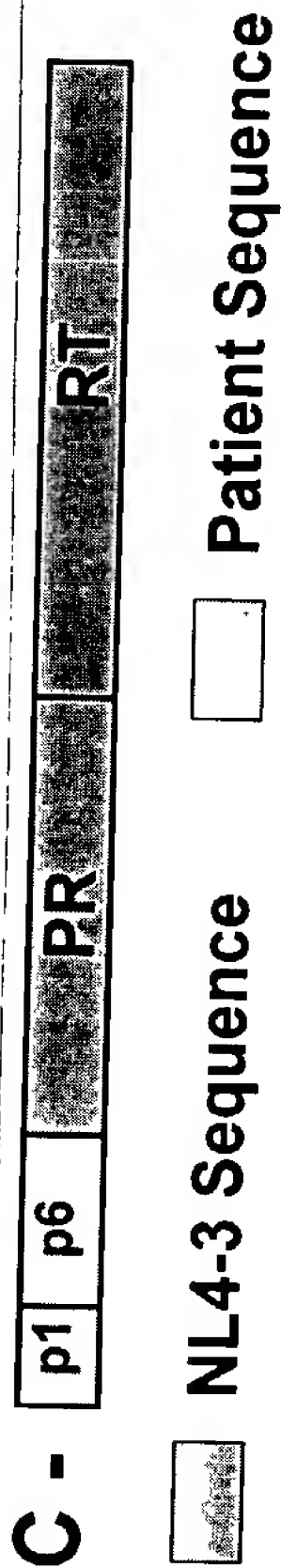
Sample	ABC	ddl	3TC	d4T	ddC	AZT	DLV	EFV	NVP	AMP	IDV	NFV	RTV	SQV
1	0.9	0.9	1.0	1.0	0.9	0.8	0.7	0.8	0.8	0.4	0.6	1.3	0.7	0.5
2	1.0	1.0	1.0	0.9	1.1	1.1	0.6	0.7	0.7	0.6	0.3	0.6	0.2	0.2
3	0.8	1.0	1.0	1.0	0.9	0.9	0.6	0.7	0.6	0.3	0.7	0.7	0.4	0.5
4	0.9	0.9	0.7	1.2	0.9	0.9	0.7	0.8	0.9	0.3	0.5	0.7	0.4	0.4
14	0.9	1.0	1.0	0.9	0.9	0.7	0.7	0.9	0.5	0.3	0.5	0.6	0.7	0.9
15	0.9	1.1	0.9	1.1	1.0	1.1	0.9	0.9	0.7	0.2	0.3	0.3	0.3	0.6
16	0.8	1.0	0.8	1.1	1.1	0.7	0.5	0.8	0.7	0.4	0.3	0.3	0.4	0.5
17	1.0	1.0	0.9	1.0	1.0	1.0	0.7	1.0	0.8	0.2	0.4	0.5	0.4	0.6
18	0.9	0.7	0.8	0.9	0.9	0.9	0.6	0.9	0.5	0.3	0.4	0.4	0.4	0.5
19	0.9	1.0	0.9	0.8	1.0	0.8	0.7	0.9	0.8	0.4	0.4	0.4	0.3	0.6
20	0.9	1.0	1.0	0.9	0.9	1.0	0.6	0.9	0.8	0.2	0.3	0.3	0.3	0.4

Replication Capacity



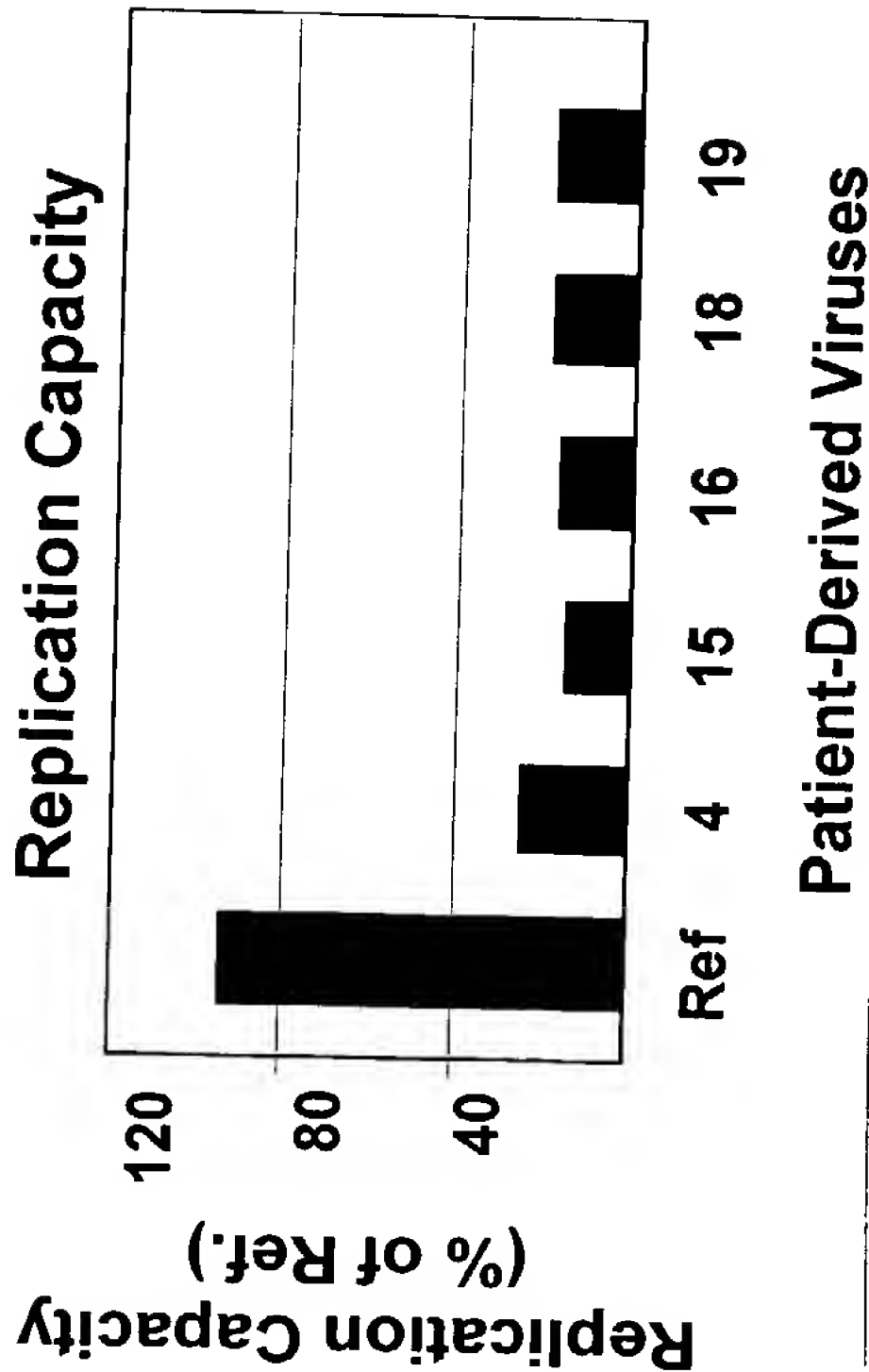
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FIGURE 13



Fold Change in Susceptibility

Sample	ABC	ddl	3TC	d4T	ddC	ZDV	DLV	EFV	NVP	AMP	IDV	NFV	RTV	SQV
4	0.9	1.0	0.9	0.9	0.7	0.8	1.1	0.7	0.6	0.6	0.5	0.6	0.6	0.4
15	0.9	1.1	1.0	1.0	0.9	0.8	1.6	0.8	0.8	0.5	0.4	0.4	0.4	0.3
16	0.8	1.0	0.9	1.0	0.9	0.8	1.3	0.7	0.6	0.3	0.4	0.3	0.3	0.5
18	0.9	0.9	1.0	1.0	0.8	0.7	1.1	0.7	0.5	0.2	0.4	0.2	0.2	0.7
19	1.0	1.0	1.0	1.0	0.9	0.7	1.1	0.7	0.5	0.3	0.3	0.3	0.3	0.5



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FIGURE 14

**What Is the Role of Sequences Flanking
the N-Terminus of PR?**

1. The Gag Frame Encodes p1 and p6

- p6 contains the L domain (PTAPP) which is critical for virus release from the cell
- p6 is required for proper incorporation of Vpr into the virions as well as retention of pol proteins
- p6 associates with TRiC (chaperonin)

2. The Pol Frame Encodes a Transframe Protein (TFR)

TFR includes a conserved octapeptide (TFP) and p6*

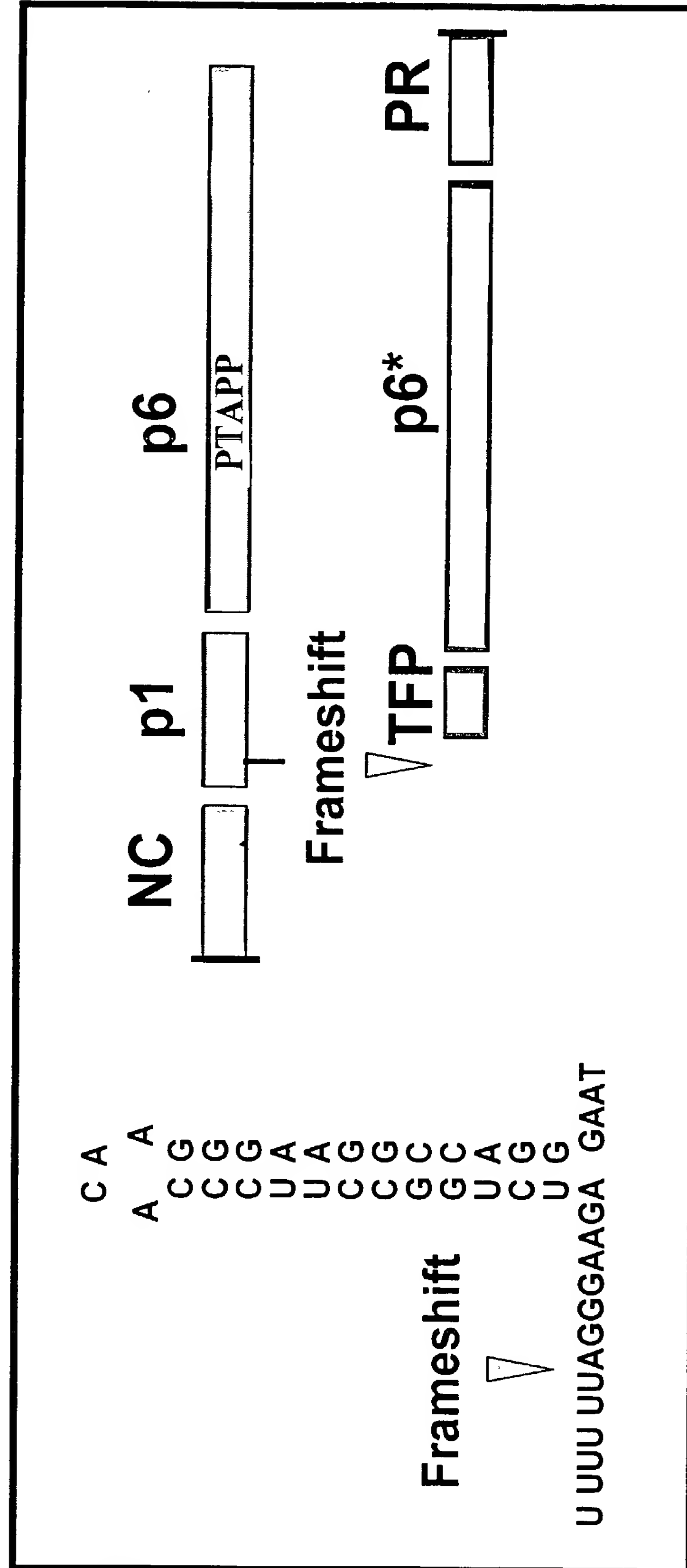
- The TFP is a potent competitive inhibitor of PR in vitro
- p6* modulates PR activity



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FIGURE 15

*Contains Sequences and Structures Required for Frameshift
Slippery heptamer sequence (U UUU UUA)
Stem loop structure downstream of the frameshift site*





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FIGURE 16
Gag p1 and p6
Genotype of Patient-Derived Sequences

ANFLGKIWP SHKGRPGN FLQSRPEPTAPPEESFRFGEE TTPSQKQEPIDKELYPLASLRSLFGNDPSSQ

IS.....N.....A.....G.....ST.....
IIV.....S.....A.....T.....K.....L.....
IIIL.....N.T.....-P.T.R.Q.....V.T.....K.....L.....
IVRS.....G.....K.....

Transframe Protein

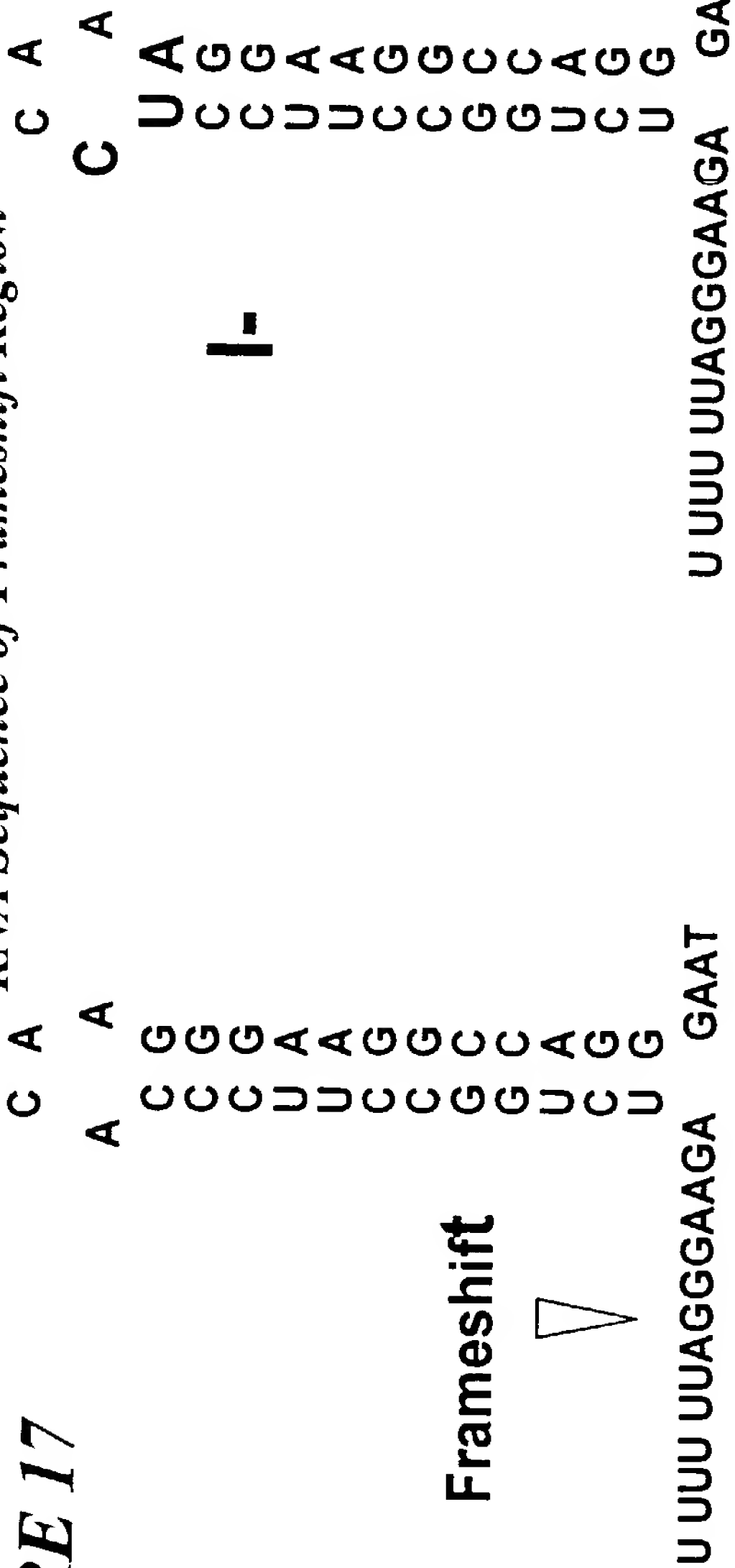
FFREDLAFPQ GKAREFSSEQ TRANSPTRRE LQWGRDNNS LSEAGADRQT VSFSEF

IL.R.....S.....N.....NL
IIN.....E..KLC...TI..S.....D.....
IIIT.....P...N.....G.....-P.D.....I..CN.
IVN.....L.R.....T.....

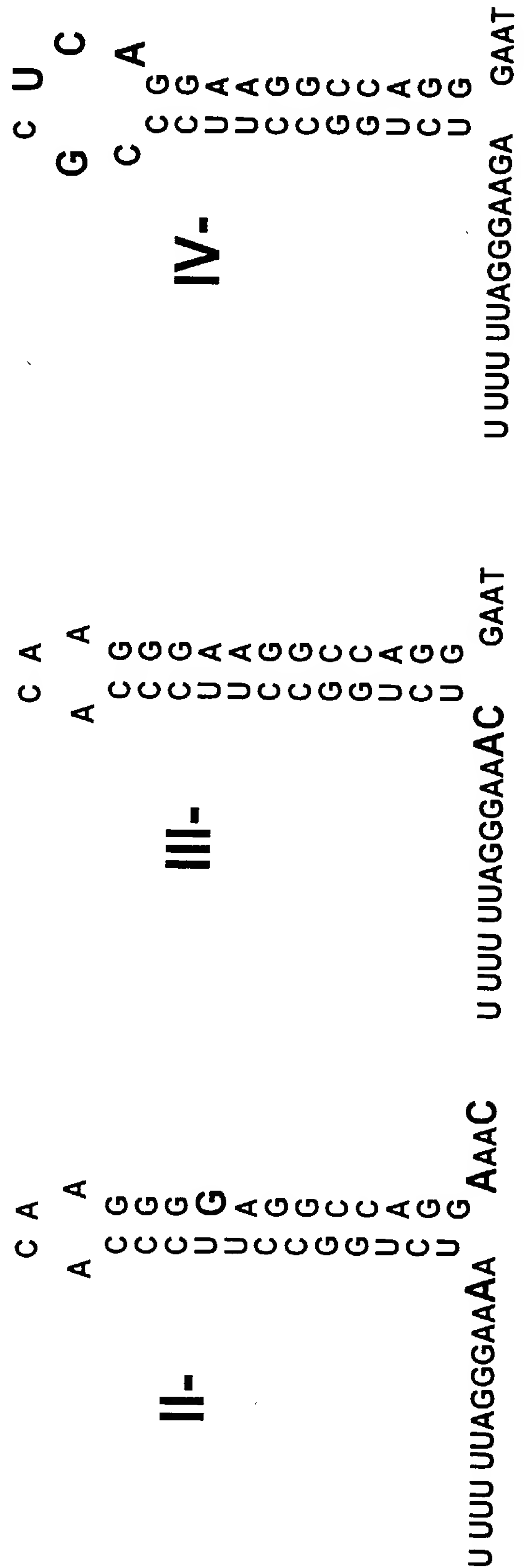
* I to IV represent clones derived from patient sample pools that retained the HS to PI

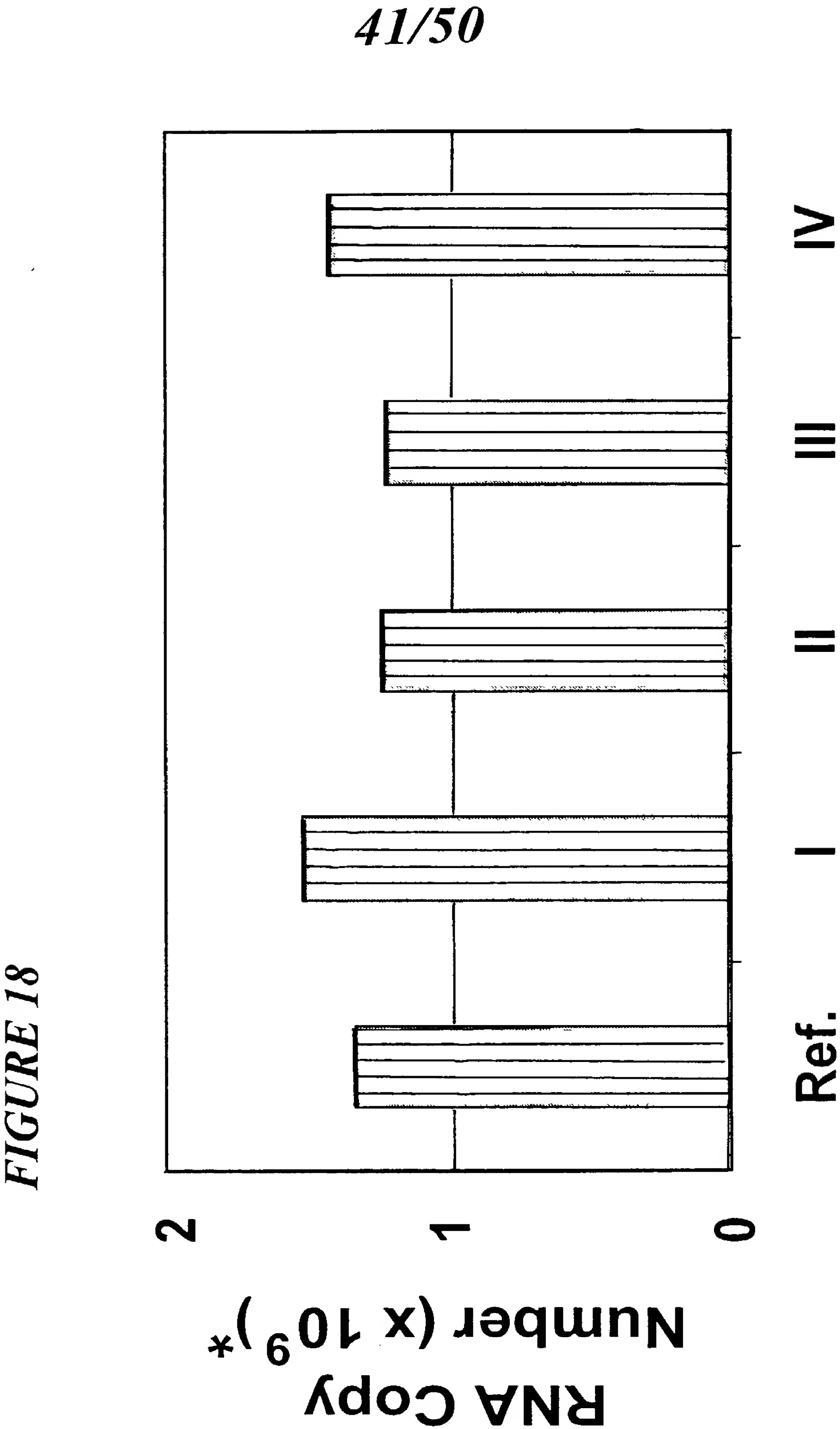
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RNA Sequence of Frameshift Region



Frameshift







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FIGURE 19

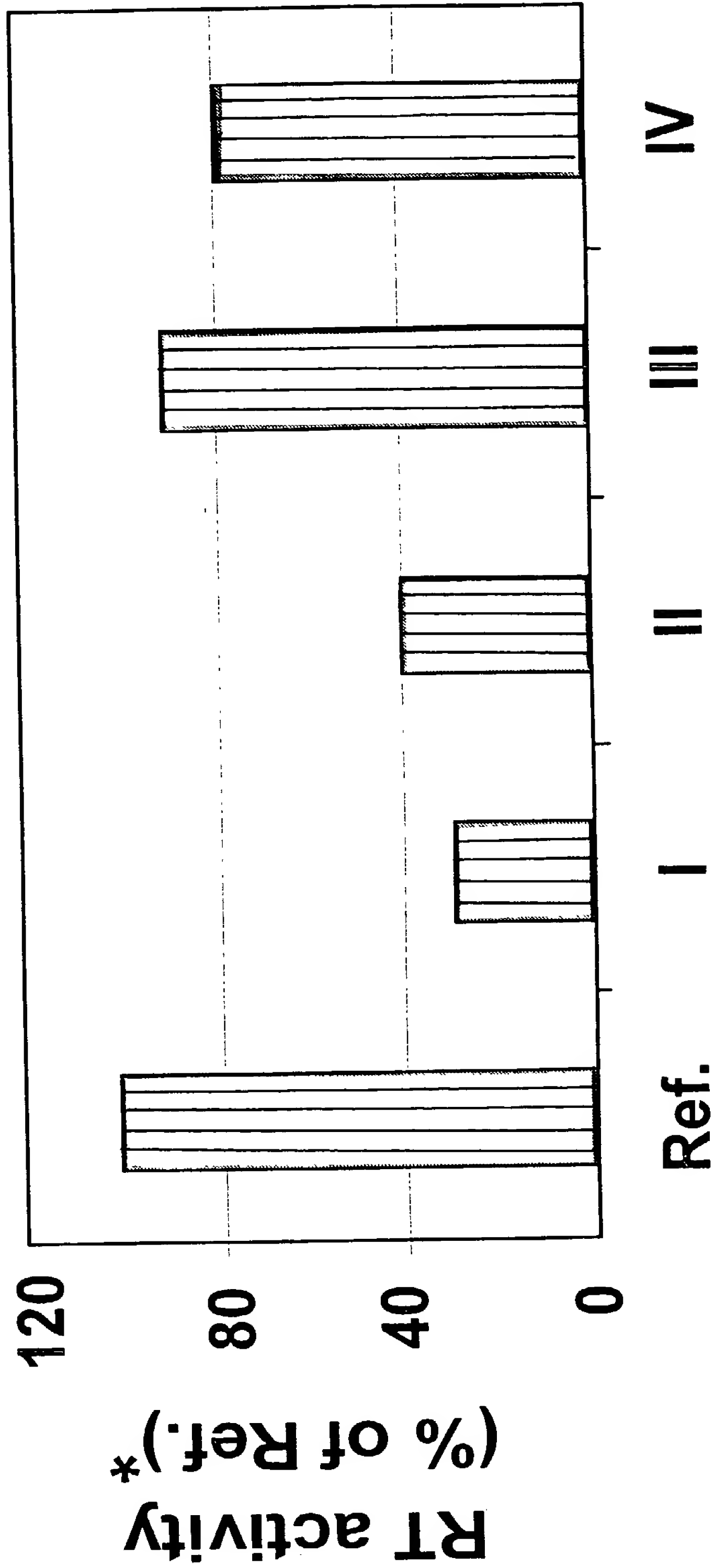
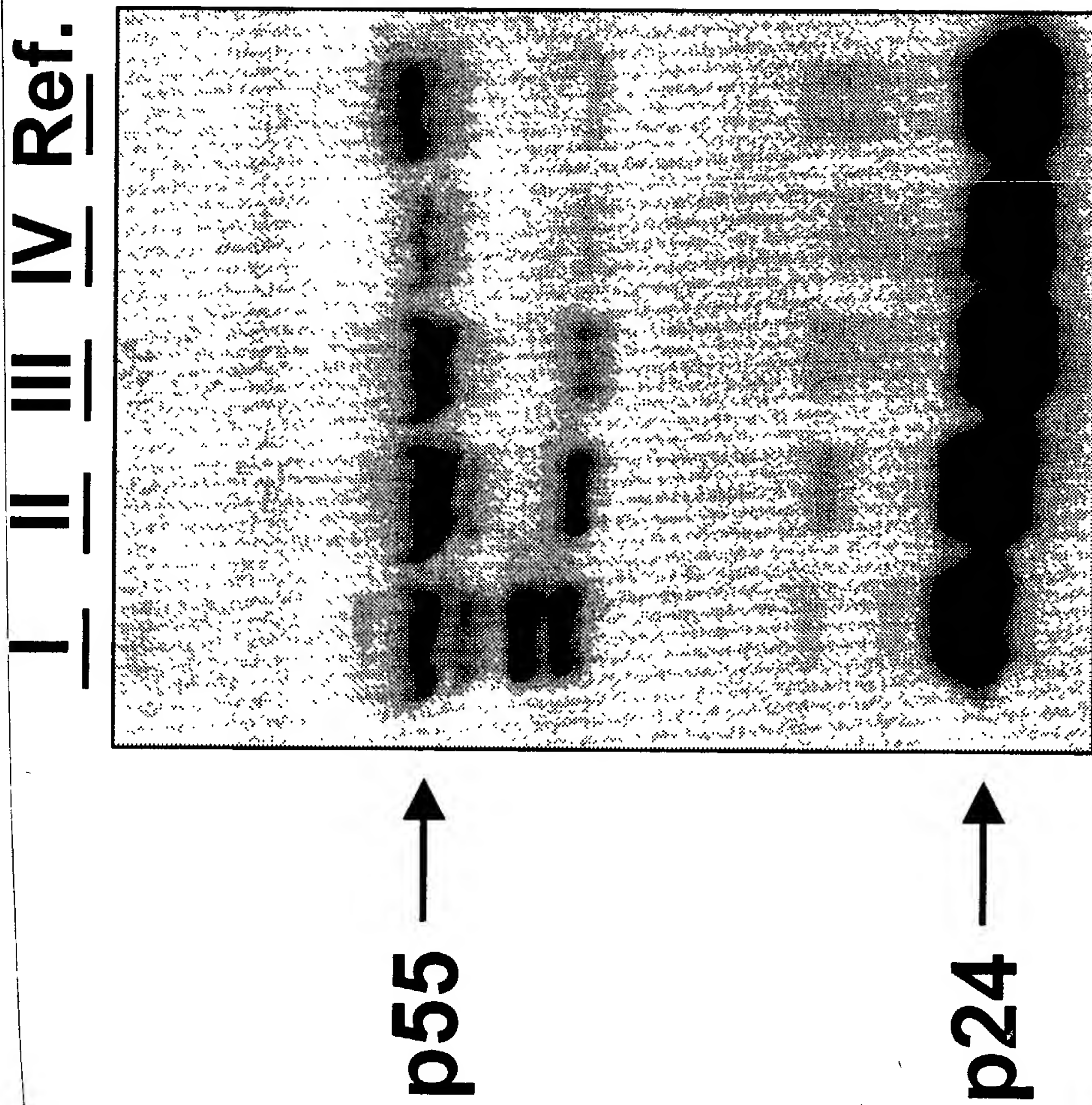


FIGURE 20

Processing of Pr55Gag in Virions
Western Blot analysis using anti-p24 antibodies



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FIGURE 21

- HS to Pls is associated with decreased viral fitness
- In 25% of the cases analyzed in this study, the HS to Pls and decreased replication capacity was attributed to mutations in gag sequences flanking the N-terminus of PR
- Genotypic analysis revealed several unusual polymorphisms in p1-p6/TFP-p6* sequences
- Recombinant viruses carrying only the C-terminal gag sequences from patient isolates that retained the HS phenotype are released efficiently from the cell. However, analysis of the virus associated RT and PR activities suggest maturation defects

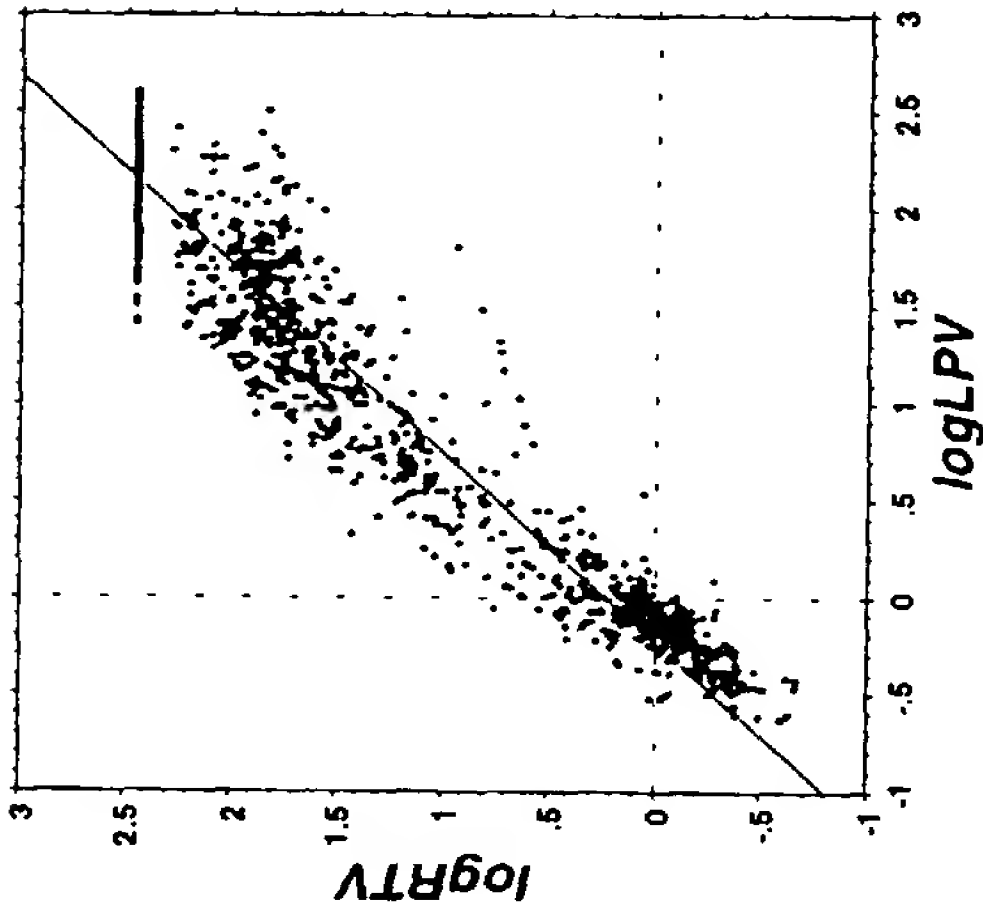


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FIGURE 22

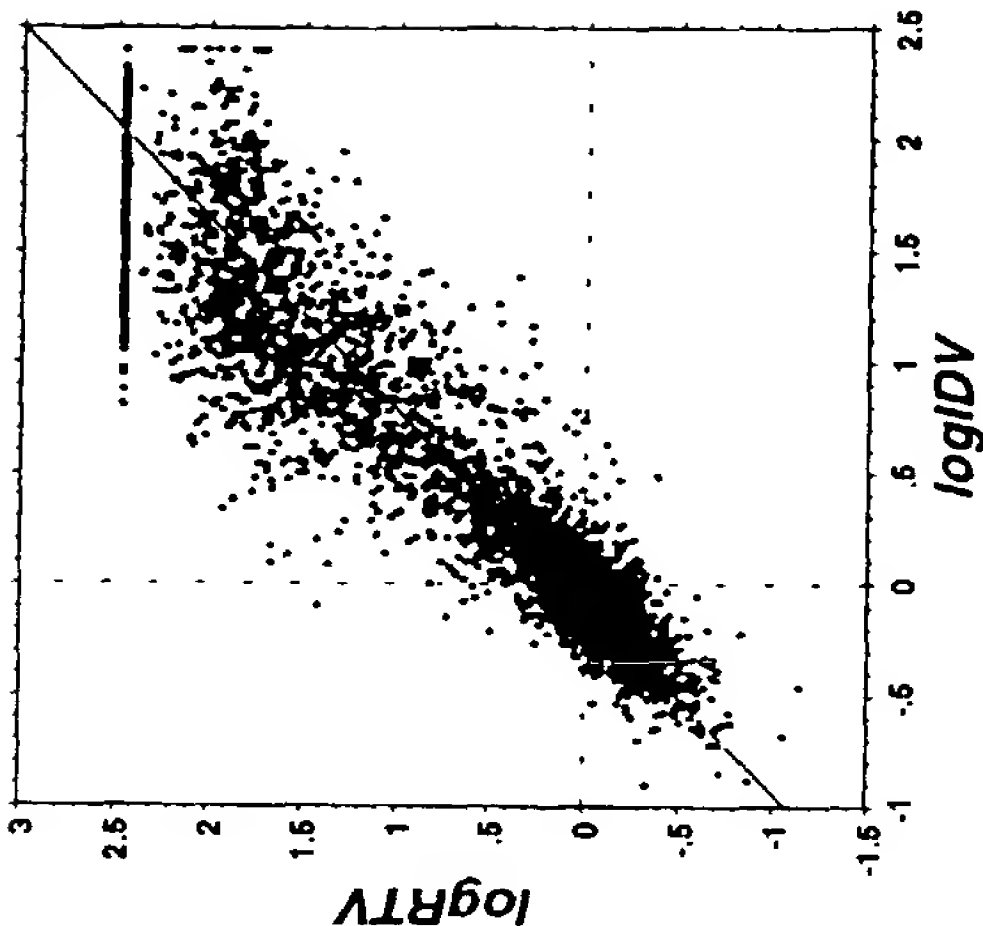
LPV vs. RTV

$R^2=0.921$



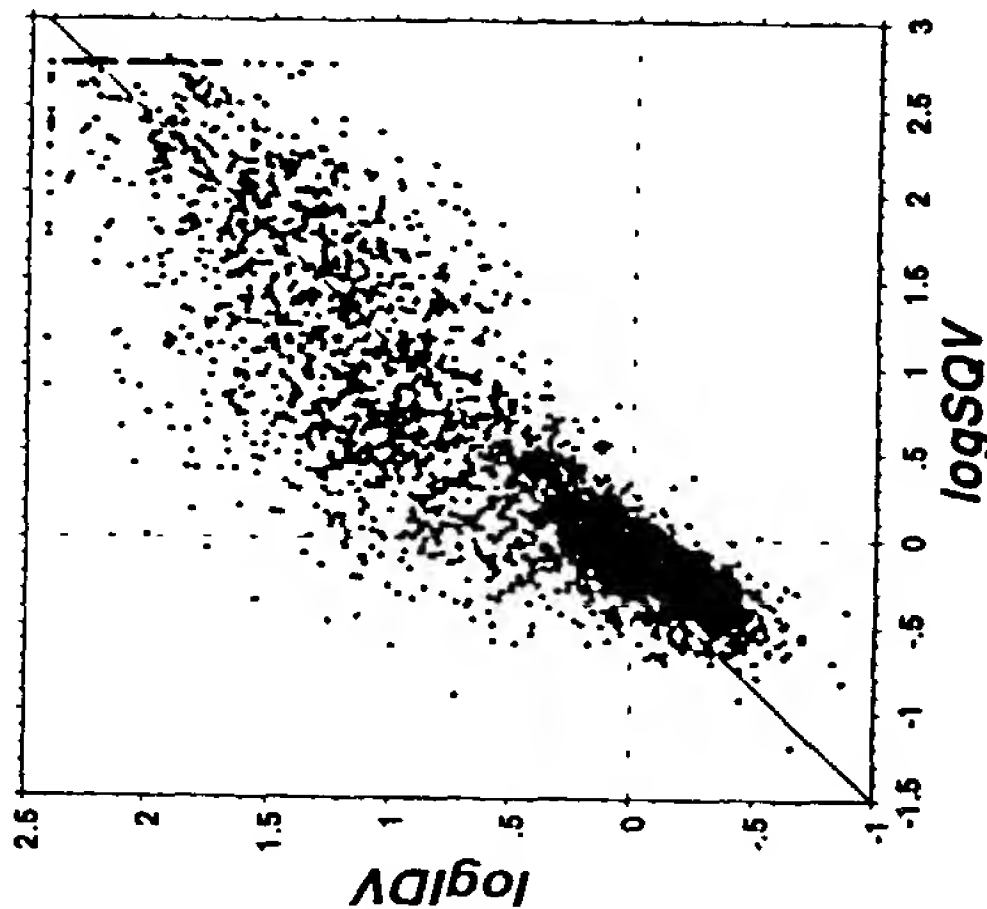
IDV vs. RTV

$R^2=0.867$



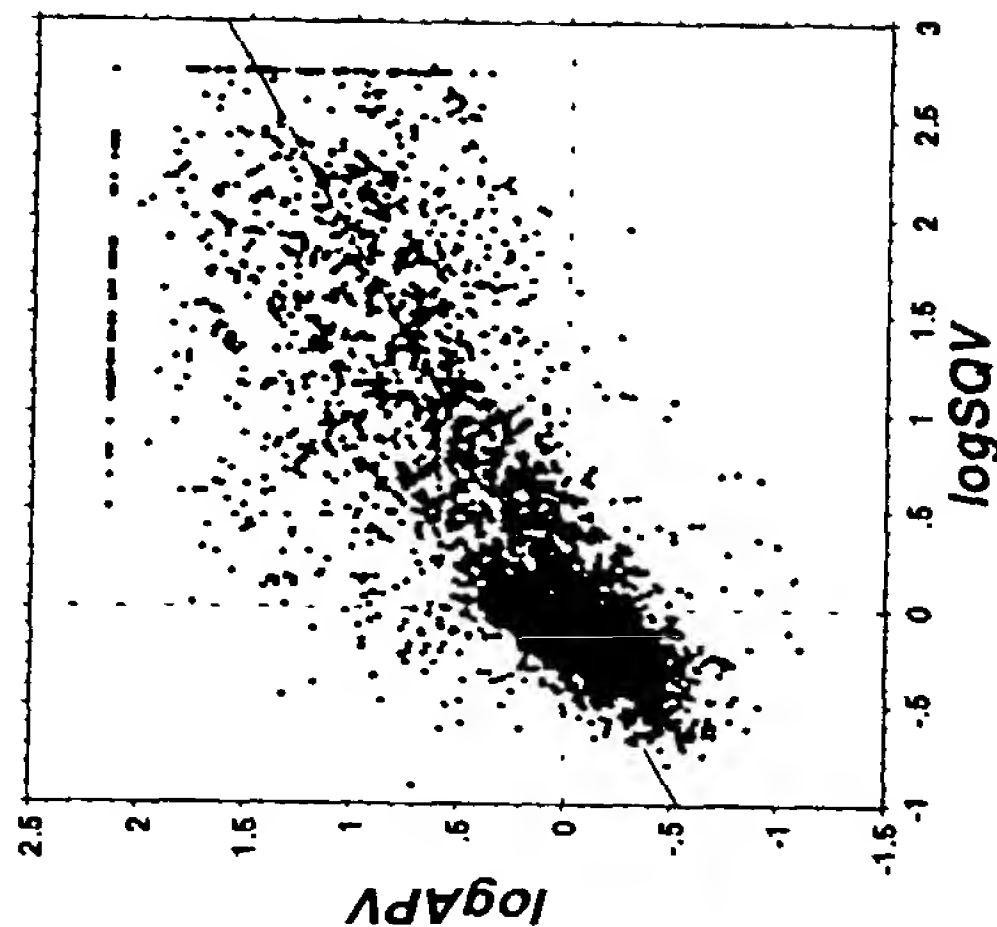
IDV vs. SQV

$R^2=0.784$



APV vs. SQV

$R^2=0.591$



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FIGURE 23

R^2 values
(sorted by drug)

R^2 values
(sorted by drug)

R^2 values (sorted by drug)			R^2 values (sorted by drug)		
PI 1	PI 2	R^2	PI 1	PI 2	R^2
APV	IDV	0.675	IDV	NFV	0.925 *
APV	LPV	0.777	RTV	LPV	0.921 **
APV	NFV	0.544	RTV	SQV	0.880 **
APV	RTV	0.737	NFV	RTV	0.873 *
APV	SQV	0.591	IDV	RTV	0.867
IDV	LPV	0.849	IDV	LPV	0.849
IDV	NFV	0.774	NFV	SQV	0.801 *
IDV	NFV	0.925 *	IDV	SQV	0.784
IDV	RTV	0.867	APV	LPV	0.777
IDV	SQV	0.784	IDV	NFV	0.774
NFV	LPV	0.757	NFV	LPV	0.757
NFV	RTV	0.696	RTV	SQV	0.740
NFV	RTV	0.873 *	APV	RTV	0.737
NFV	SQV	0.691	NFV	RTV	0.696
NFV	SQV	0.801 *	NFV	SQV	0.691
RTV	LPV	0.921	SQV	LPV	0.678
RTV	SQV	0.740	APV	IDV	0.675
RTV	SQV	0.880 **	APV	SQV	0.591
SQV	LPV	0.678	APV	NFV	0.544

R^2 values for pairwise comparisons (all samples)

APV	1	0.675	0.777	0.544	0.737	0.591
IDV	0.675	1	0.849	0.774	0.867	0.784
LPV	0.777	0.849	1	0.757	0.921	0.678
NFV	0.544	0.774	0.757	1	0.696	0.691
RTV	0.737	0.867	0.921	0.696	1	0.740
SQV	0.591	0.784	0.678	0.691	0.740	1

<0.7

0.7-0.8

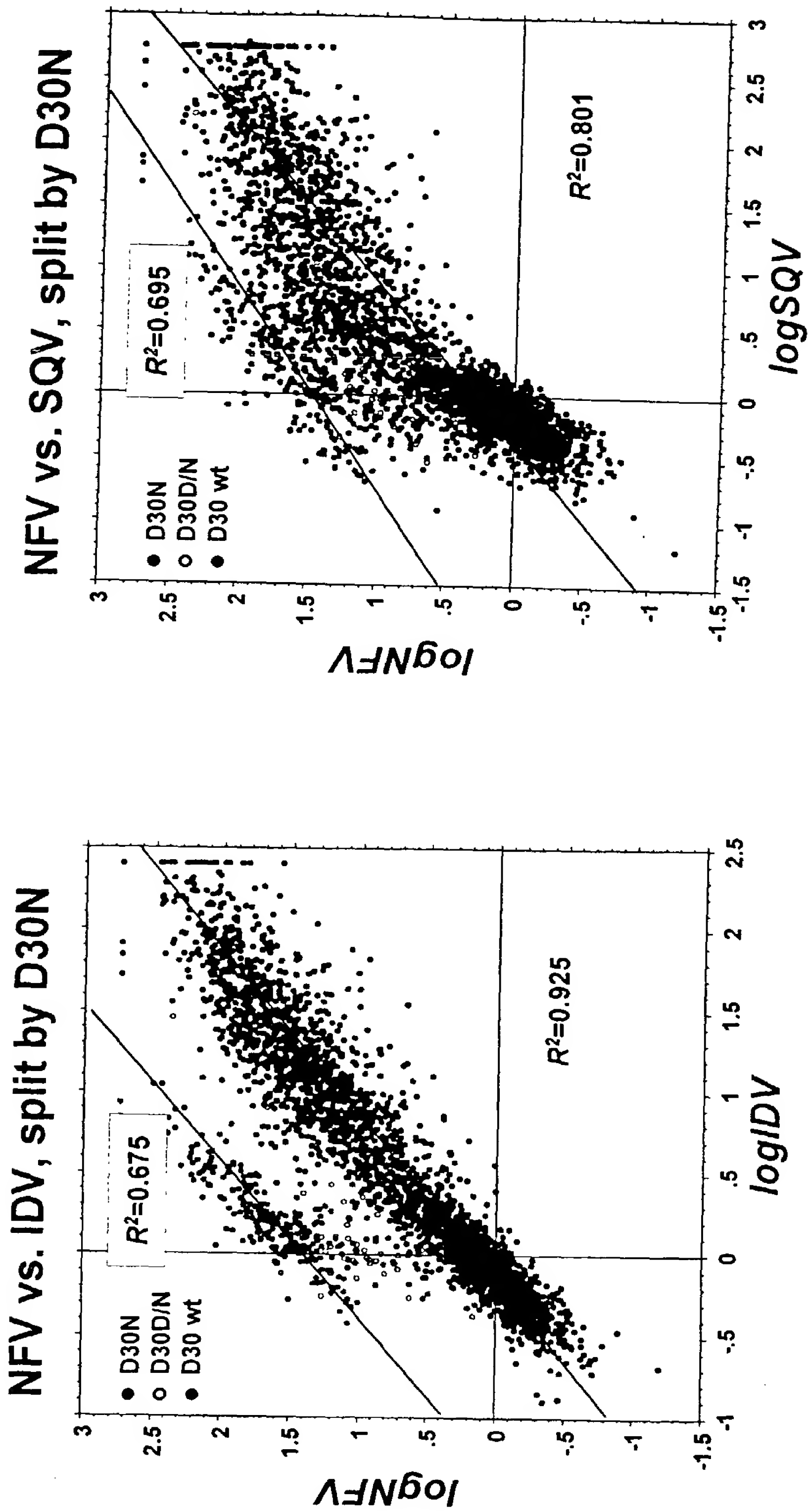
0.8-0.9

>0.9

* Excluding viruses with D30N (see Fig.4)

** Excluding viruses with V82AFST (see Fig.5)

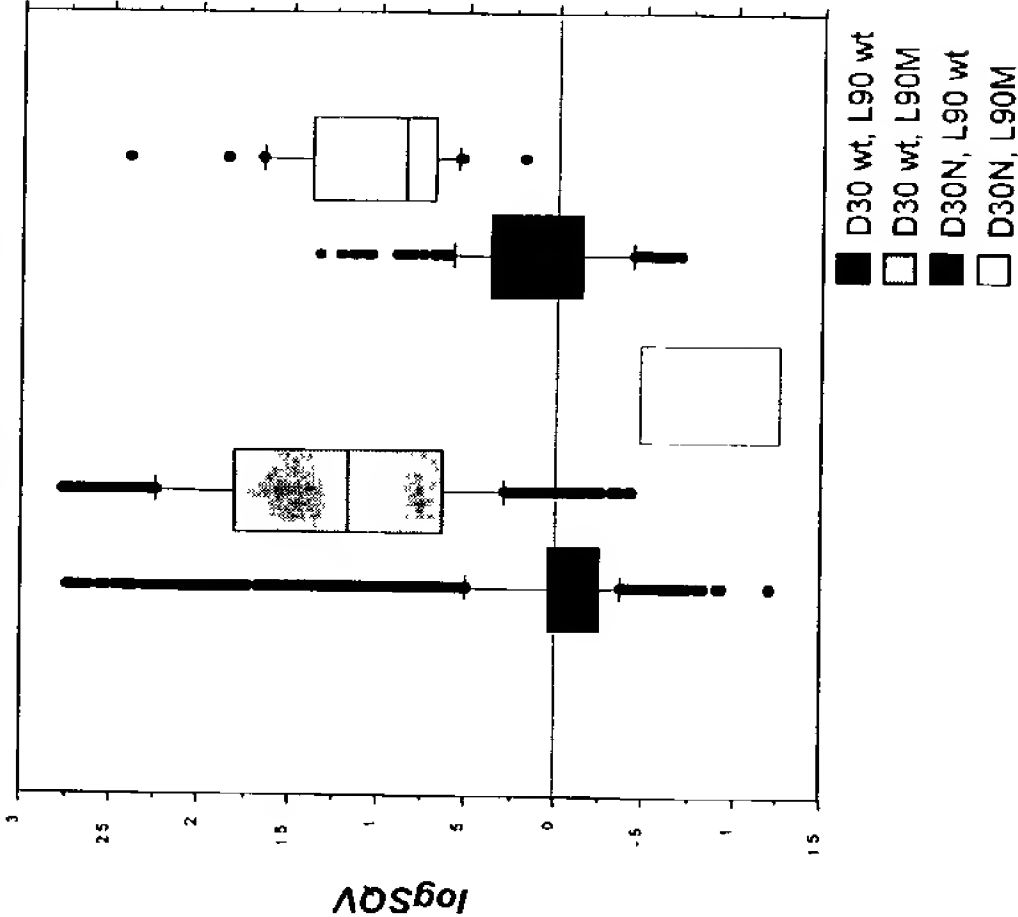
FIGURE 25



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FIGURE 26

SQV fold change +/- D30N, L90M



Phenotypes of samples containing D30N, and/or L90M, from the database (boxes contain a bar at the median and represent the 25th to 75th percentiles; the error bars represent the 10th and 90th percentiles; and the dots are the outliers.

D30N/N88D/L90M: Patient samples

PR genotype (resistance-associated mutations)	Fold-change in IC ₅₀ vs. reference				
	AMP	IDV	NFV	RTV	SQV
L10L/V, D30N, L33L/F, M36I, L63P, A71T, N88D, L90M	1.9	2.8	160.4	8.2	9.6
D30N, L63P, V77I, N88D, L90M	1.3	3.2	74.2	4.0	7.1
D30N, M36I, L63P, A71T, N88D, L90M	1.1	2.6	124.0	3.6	4.4
D30N, L63P, V77I, N88D, L90M	2.0	5.3	57.0	3.4	9.3
L10F, D30N, L33F, I54L, L63P, A71V, V77I, N88D, L90M	11.4	1.1	103.8	4.7	6.1
D30N, L63P, V77I, N88D, L90M	3.7	3.9	171.4	5.7	38.1
L10F, D30N, I54L, L63P, A71T, V77I, N88D, L90M	0.4	1.3	32.8	2.1	3.7
D30N, L63P, V77I, N88D, L90M	2.3	7.6	217.5	3.9	11.9
L10L/R, D30N, M36I, I54L/L, L63P, A71V, N88D, L90M	2.7	5.2	140.1	10.2	21.0
D30N, M36I, I54V, L63P, A71V, N88D, L90M	1.5	5.8	218.5	16.8	24.3
K20K/R, D30N, M36I, F53F/L, I54V, L63P, A71V, N88D, L90M	2.3	8.4	>550	35.0	72.0
L10L/F, I13V, L19T, D30N, R41K, L63P, N88D, L90M	1.2	1.7	46.9	2.5	5.0
D30N, L63P, V77I, N88D, L90M	1.0	2.3	66.8	3.1	3.9
L10F, K20T, D30N, L33F, M36I, M46M/I, I54L, L63P, A71V, V77I, N88D, L90M	27.6	6.8	>550	31.2	45.3
D30N, L33F, L63P, A71A/T, N88D, L90M	1.3	1.3	35.7	2.7	3.5
D30N, L63P, V77I, N88D, L90M	1.5	3.5	73.7	3.3	5.2
D30N, M36I, I54V, L63P, A71V, N88D, L90M	2.2	12.0	140.4	27.0	45.8
L10F, K20R, D30N, V32V/I, L33L/F/I, M36I, M46I, I47I/V, I54V/A/M/T/V, L63P, A71V, V82V/A, N88D, L90M	>130	>250	>550	>275	257.5

2.5-10 fold >10 fold

Phenotypes of samples containing D30N, N88D, and L90M. There are no mixtures detected at these sites, indicating that the mutations are linked. All have reduced susceptibility (>2.5-fold change in IC₅₀) to NFV and SQV.



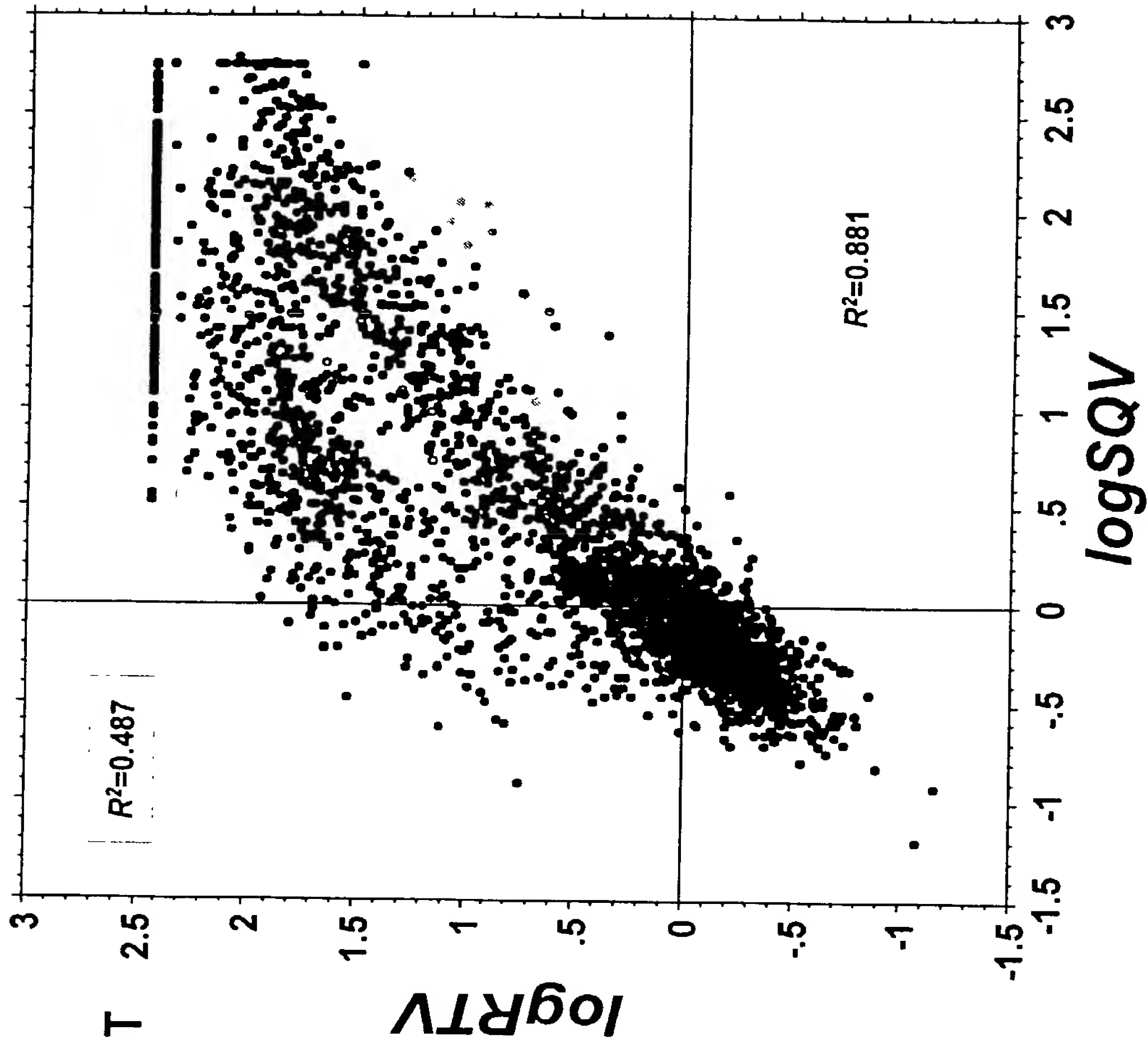
Applicants : Neil T. Parkin and Rainer Ziermann
U. S. Serial No. 09/874,472
Filing Date: June 4, 2001
Title of the Invention: MEANS AND METHODS FOR
MONITORING PROTEASE INHIBITOR
ANTIRETROVIRAL THERAPY AND GUIDING
THERAPEUTIC DECISIONS IN THE TREATMENT
OF HIV/AIDS

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FIGURE 27

SQV vs. RTV,
split by V82AFST
and G48V



- V82AFST, G48 wt
- G48V, V82 wt
- G48V, V82AFST
- G48 wt, V82 wt



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FIGURE 28

